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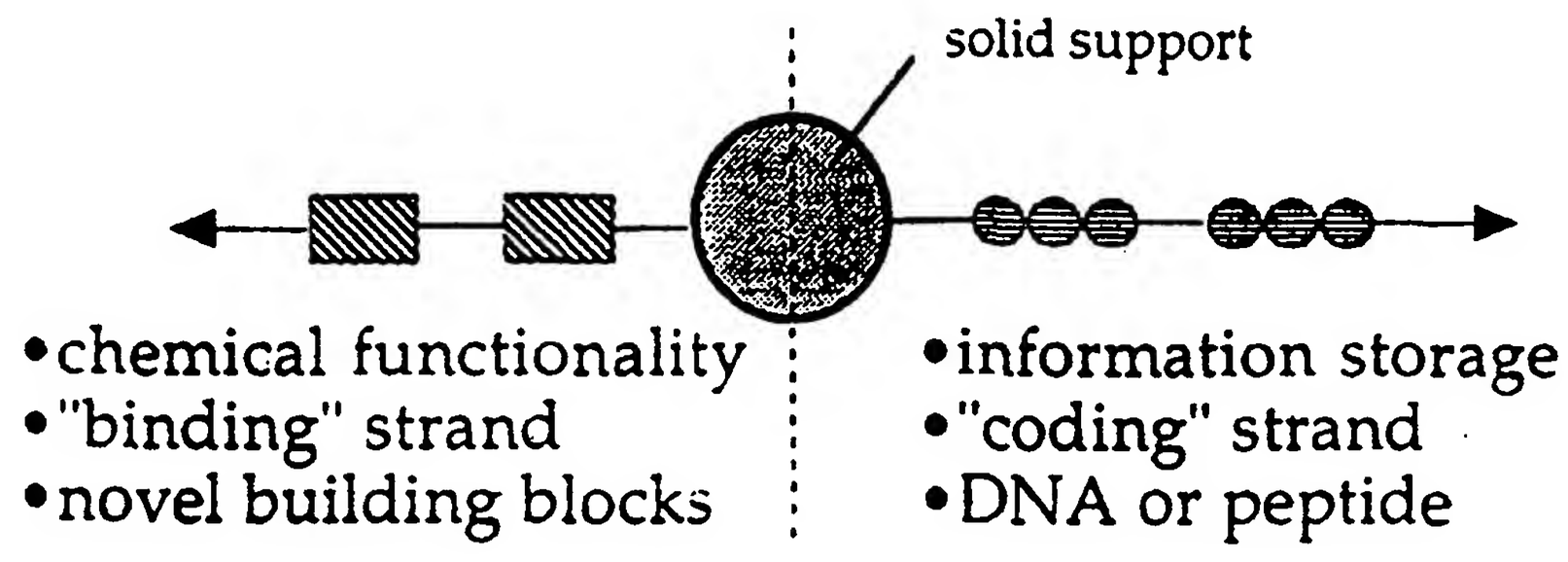

(57) Abstract

Conjugates and methods of producing the conjugates and mixtures thereof are disclosed. Conjugates are comprised of an active polymer made up of monomer units selected from the group consisting of the monomer units of peptides and/or peptoids, and an encoding polymer comprised of encoding monomers wherein the encoding polymer corresponds to the active polymer, and a coupling moiety covalently coupled to the active polymer and the encoding polymer. In accordance with the synthesis methodology, mixtures of large numbers of conjugates are produced by providing a coupling moiety and covalent binding it to an active monomer and an encoding monomer. Additional monomer units are added to the active monomer to create an active polymer and additional monomer units are added to the encoding monomer to produce an encoding polymer until the desired length is reached for the active polymer. Mixtures of conjugates attached to support bases can be used to assay a sample. The sample is brought into contact with the conjugates and a determination is made with respect to which active proteins bind to receptor sites within the sample. When active binding proteins are determined, the encoding polymer associated with the active polymer is sequenced, and by deduction, the sequence of the active polymer is determined.

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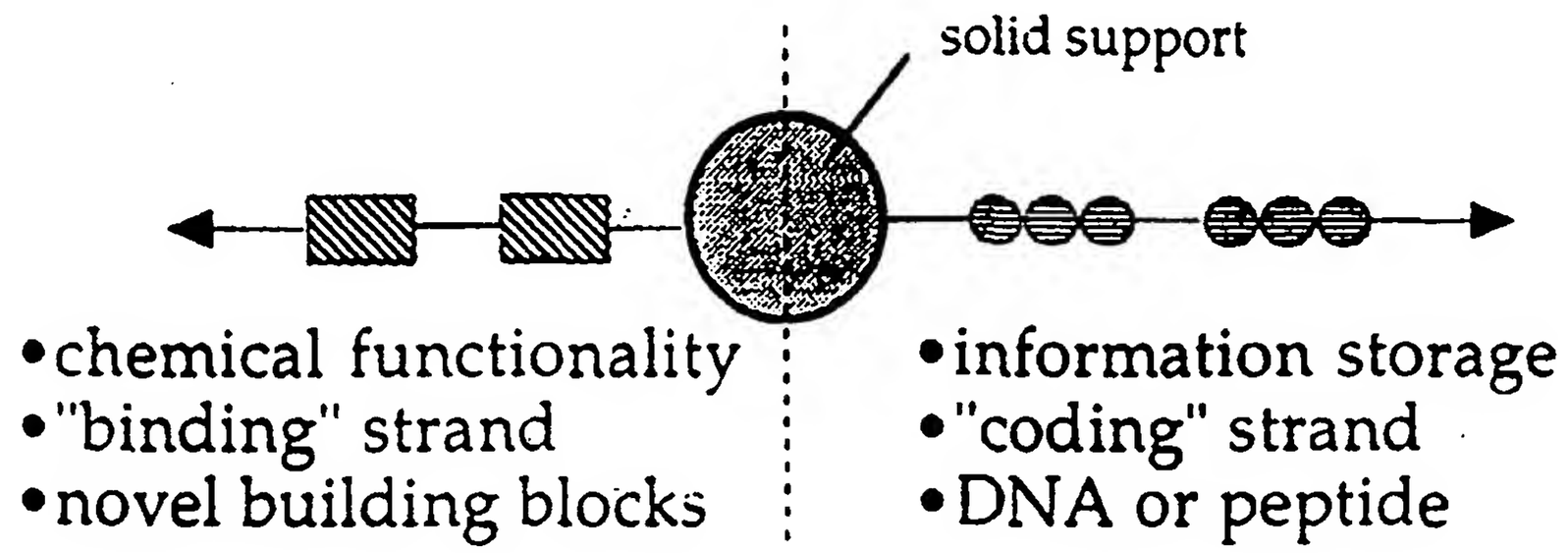

(57) Abstract

Conjugates and methods of producing the conjugates and mixtures thereof are disclosed. Conjugates are comprised of an active polymer made up of monomer units selected from the group consisting of the monomer units of peptides and/or peptoids, and an encoding polymer comprised of encoding monomers wherein the encoding polymer corresponds to the active polymer, and a coupling moiety covalently coupled to the active polymer and the encoding polymer. In accordance with the synthesis methodology, mixtures of large numbers of conjugates are produced by providing a coupling moiety and covalent binding it to an active monomer and an encoding monomer. Additional monomer units are added to the active monomer to create an active polymer and additional monomer units are added to the encoding monomer to produce an encoding polymer until the desired length is reached for the active polymer. Mixtures of conjugates attached to support bases can be used to assay a sample. The sample is brought into contact with the conjugates and a determination is made with respect to which active proteins bind to receptor sites within the sample. When active binding proteins are determined, the encoding polymer associated with the active polymer is sequenced, and by deduction, the sequence of the active polymer is determined.

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SYNTHESIS OF ENCODED POLYMERS

Field of the Invention

This invention relates to the fields of biopolymer synthesis and drug design. More particularly, the invention relates to methods for synthesizing libraries of biologically active polymers in association with an included polymer which is encoded to facilitate deciphering.

Background of the Invention

Modern pharmaceutical technology has taken two divergent paths in pursuit of new therapeutic compounds. Rational drug design achieves results by intensive analysis of the molecular structure of binding sites, and designing compounds specifically to complement a desired binding site. For example, one interested in preparing new antihypertensive compounds might analyze the molecular structure of the β-adrenergic receptor binding site using X-ray crystallography and/or advanced NMR techniques, and then synthesize compounds calculated to fit within the binding site and complement the charge distribution.

The other approach is to prepare an enormous library of compounds and select only those compounds which exhibit a desired activity. This approach differs from the traditional pharmaceutical cycle of design/synthesize/test/synthesize variants by conducting the screening step in a massively parallel fashion, screening an enormous number of different compounds simultaneously. The challenge to this

approach is first to provide a group of compounds for screening that is sufficiently numerous and diverse to insure that the activity sought is represented in the group, and second to identify the active compounds at low concentration within the group.

Rutter et al., US 5,010,175 disclosed a method of making diverse mixtures of peptides by adjusting the concentration of each activated peptide in proportion to its reaction rate, in order to obtain a substantially equimolar mixture of peptides. Rutter also disclosed the process of providing a mixture of peptides (having at least 50 different peptides), and selecting one or more peptides having a desired property and separating them from the rest of the peptides.

Zuckermann et al., PCT WO91/17823 disclosed an alternative method for preparing diverse mixtures of oligopeptides on solid-phase resins, and a robotic device for performing the necessary manipulations. In this method, a pool of resin particles is separated into a number of groups (wherein each group is defined as one or more separate reactions), and a different amino acid coupled to the resin in each group. The groups are then mixed together, separated into a number of groups, and again coupled with a different amino acid for each group. This cycle is repeated until the desired number of amino acids per oligopeptide is obtained. One advantage of this approach is that each coupling reaction occurs in isolation from other reactants, which permits one to drive each reaction to completion without carefully adjusting the initial concentration of each reactant. This method also facilitates the preparation of oligopeptides wherein some positions within the peptide chain are held constant, and where some positions are restricted to less than all amino acids. For example, one may use this method to prepare a peptide of the formula X_1-X_2-X_3-Glu-Ala-X_4-X_5-X_6, where X_n

can be any amino acid. If desired, one could limit, for example, X_3 and X_5 to hydrophobic residues. Zuckermann also disclosed that this method may be applied to the synthesis of oligonucleotides, which may then be inserted into cloning and expression vectors for biological expression.

Bartlett et al., PCT WO91/19735 disclosed a variation of the Zuckermann et al. method in which a diverse set of non-amino acid monomers is employed to form mixtures of compounds called "peptoids." Peptoids sample a different region of physico-chemical parameter space than traditional oligopeptides, depending on the type of linkage between monomers, and may be able to exhibit activities unavailable to peptide libraries due to the diversity (or difference) in side chains.

Houghten, US 4,631,211 disclosed a "tea-bag" peptide synthesis method. The "tea bags" are mesh bags containing resin beads for peptide synthesis. Houghten's method enables one to add the same amino acid to a number of different oligopeptides without mixing the products: a number of "tea bags" may be reacted with an amino acid in a common pot, then separated physically.

Cook, EP 383620 described synthesis of COP-1, a random polymer of Ala, Glu, Lys, and Tyr, having an average molecular weight of 23 kDa having activity in the treatment of multiple sclerosis. COP-1 is made in the prior art by chemical polymerization of the amino acids. However, Cook described expression from genes made by random polymerization of oligonucleotides, and selection for those clones expressing COP-1 with the highest activity.

Lebl et al., EP 445915 described a machine for performing multiple simultaneous peptide syntheses using a planar support surface. The planar support is, for example, paper or cotton.

Kauffman et al., WO86/05803 disclosed production of peptide libraries by expression from synthetic genes which are partially or wholly "stochastic." Stochastic genes are prepared by polymerizing a mixture of at least three oligonucleotides (at least heptamers) to form a double-stranded stochastic sequence, and ligating the stochastic sequence into an expression vector.

Lam et al., WO92/00091 disclosed libraries of oligonucleotides, oligopeptides, and peptide/nucleotide chimeras, and methods for screening the libraries for active compounds. However, Lam did not disclose conjugates having an active sequence and a coding sequence.

K.M. Derbyshire et al., Gene (1986) 46:145-52 disclosed a method for "saturation mutagenesis" of a segment of DNA, by synthesizing oligonucleotides using contaminated pools of monomer. Each A, C, G, and T reservoir contained 1/54 parts of each of the other bases. The object was to prepare a DNA segment mixture having one or two mutations per sequence. They did not observe equal frequencies of mutation, presumably due to differences in coupling efficiency. The authors suggested synthesizing sequences using four reservoirs containing pure bases, and one reservoir containing a mixture of all four bases in the concentrations necessary to balance the coupling efficiencies.

J.F. Reidhaar-Olson et al., Science (1988) 241:53-57 disclosed the generation of mutant λ repressor proteins by replacing two codons with random nucleotides (NNG/C). The resulting mutant proteins were assayed for activity to determine which amino acid positions were critical, and which positions should be conserved.

I.S. Dunn et al., Prot Eng (1988) 2:283-91 disclosed the use of random polynucleotides to generate mutant β-lactamase α-peptides, some of which

exhibited properties superior to the native sequence α-peptide.

A.R. Oliphant & K. Struhl, Nuc Acids Res (1988) 16:7673-83 disclosed the use of random polynucleotides to investigate promoter function. A section of random polynucleotide was inserted into the -35 to -10 region of a gene conferring drug resistance in E. coli, and the transformants screened for resistance. Survivors were cloned and sequenced to provide a functional consensus sequence.

F.W. Studier, Proc Natl Acad Sci USA (1989) 86:6917-21 disclosed a method for sequencing large volumes of DNA by random priming of cosmid libraries.

A.R. Oliphant et al., Proc Natl Acad Sci USA (1989) 86:9094-98 disclosed the generation of β-lactamase mutants having altered properties, by cloning a random polynucleotide into the β-lactamase gene.

D.K. Dube et al., Biochem (1989) 28:5703-07 disclosed the generation of β-lactamase mutants having altered properties, by cloning a random polynucleotide into the β-lactamase gene.

R.A. Owens et al., Biochem Biophys Res Comm (1991) 181:402-08 disclosed the selection of an HIV protease inhibitor from a library of 240,000 tetrapeptides (in 22 mixtures). The mixtures were prepared by the "mixed resin" technique.

These techniques enable one to prepare libraries of diverse compounds. However, the problem of identifying the resulting compounds has seldom been addressed. Oligopeptides are typically sequenced by stepwise cleavage of each amino acid from the parent compound (which is usually immobilized on a resin), with chromatographic analysis of the cleaved moiety. Sensitive techniques are required to distinguish between twenty or more amino acids. Analysis is further complicated when uncommon amino acids are

employed (using current techniques), especially when monomers are linked without using amide bonds.

Summary of the Invention

The present invention provides a method of synthesizing true mixtures of diverse oligopeptides and/or peptide-like compounds along with an associated encoding polymer making it possible to easily analyze those compounds exhibiting a desired activity. The invention involves synthesizing an encoding DNA strand simultaneously with the peptide/peptoid. Each unique peptide/peptoid sequence associated with its own unique DNA strand to provide the conjugates of the invention. These conjugates are screened to determine which peptide/
peptoid compounds exhibit a desired activity, and the active conjugates analyzed by DNA sequencing methods to determine the attached peptide/peptoid sequence by deduc-tion, i.e., since each DNA sequence is associated with a known peptide/peptoid, once the DNA sequence is deter-
mined, the sequence of the peptide/peptoid can be deduced.

Another aspect of the invention is a conjugate comprising a peptide or peptoid coupled to and/or directly associated with a coding polymer (CP), e.g. a nucleic acid (NA). The peptide/peptoid/CP conjugate may be linked directly (i.e., covalently bound either directly or through a small organic mol-
ecule), or by linkage to the same support (e.g., by synthesizing both peptide/peptoid and CP strand on the same particle or bead of resin).

An important object of the invention is to provide a chemical synthesis method which allows the production of libraries of peptides and/or peptoids along with a unique encoded polymer such as a DNA strand which makes it possible to readily determine the sequence of the peptide or peptoid.

An advantage of the present invention is that the methodology makes it possible to readily identify and sequence peptides and/or peptoids having desirable biological activities.

A feature of the present invention is that sequences of peptoids or peptides which contain nonconventional amino acids can still be readily determined by sequencing associated polymers such as DNA sequences which are simultaneously synthesized with the peptoids and encode them.

These and other objects, advantages and features of the present invention will become apparent to those persons skilled in the art upon reading the details of the structure, synthesis and use as more fully set forth below, reference being made to the accompanying figures forming a part hereof.

Brief Description of the Drawings

Figure 1 is a schematic diagram showing a specific embodiment of a conjugate of the invention which conjugate includes a "binding" strand or active polymer attached to a solid-support substrate which substrate is also bound to an information storage or "coding" strand;

Figure 2 is a schematic flow diagram demonstrating how encoded libraries can be synthesized on beads as the solid-support substrate;

Figure 3 is a schematic diagram showing methods of the synthesis of both solid-phase and solution-phase libraries;

Figure 4 is a schematic diagram showing resin-bound libraries generated by the derivatization of non-hydrolyzable resins;

Figure 5 is an HPLC chromatogram of binding and coding peptide strands simultaneously synthesized via non-hydrolyzable resin linkage;

Figure 6 is an HPLC chromatogram of a coding and binding strand adduct which was synthesized via a hydrolyzable resin linker;

Figure 7 is a plotted graph resulting from ELISA competition of binding sequences versus binding/encoding sequences;

Figure 8 is a schematic diagram showing the analysis of a solid-phase amptide; and

Figure 9 is a schematic flow diagram showing the analysis of a solution-phase amptide.

Detailed Description of Preferred Embodiments

Before the present method of synthesis, conjugates and methods of using such are described, it is to be understood that this invention is not limited to the particular methodologies, conjugates, or methods of use described as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting since the scope of the present invention will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a," "and," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a peptide" includes mixtures of peptides, reference to "an amino acid" includes mixtures thereof, and reference to "the reaction" includes one or more reactions of the same type as generally understood by those skilled in the art, and so forth.

Unless defined all otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or

testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to describe and disclose specific information for which the reference was cited in connection with.

In general, the invention provides a rapid method of synthesizing large numbers of conjugates which conjugates are comprised of a peptide/peptoid sequence, e.g., an amino acid sequence associated with a unique encoding sequence, e.g., a DNA sequence. The conjugates can be readily synthesized and thereafter screened for biological activity, and when activity is found, the particular peptide/peptoid sequence found to be active can be readily identified by its associated encoding (DNA) strand. Each conjugate of the invention is comprised of at least two components with one of the components being the peptide or peptoid sequence which binds to a receptor of interest and the other sequence being a polymer which encodes the binding sequence. The invention may utilize standard amino acids and DNA as encoding monomers to produce a chemically diverse library of solution-phase or solid-phase conjugates. In order to further describe the invention in detail, the following definitions are provided.

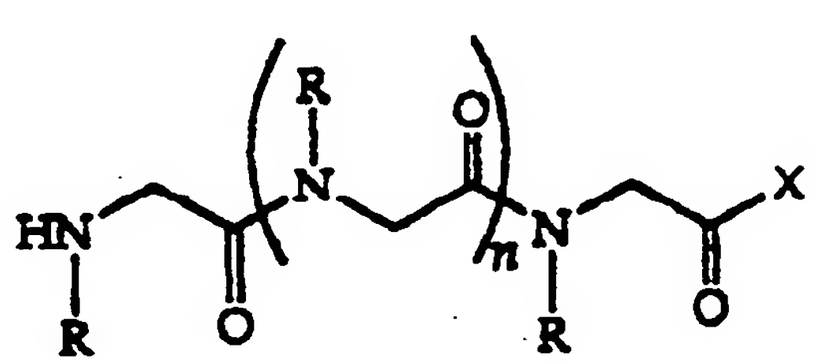
A. Definitions

The terms "nucleic acid" and "NA" refer to oligomers constructed from DNA and/or RNA bases which may be sequenced using standard DNA sequencing techniques. The NAs used herein may include uncommon bases so long as such bases are distinguishable from the other bases employed under the DNA sequencing methods to be used and include peptide-nucleic acids (PNAs) (disclosed by Nielsen, P.E., Egholm, M., Berg, R.H. & Buchardt, O., Science (1991) 254, 1497-1500). Such PNAs could serve as coding strands and the

detection would be by hybridi-zation. NAs will usually be constructed from monomers linked by phosphodiester bonds, but other similar linkages may be substituted if desired. For example, phosphorothioates may be employed to reduce lability.

The term "peptide" as used herein refers to the 20 commonly-occurring amino acids: alanine (A), cysteine (C), aspartic acid (D), glutamic acid (E), phenylalanine (F), glycine (G), histidine (H), isoleucine (I), lysine (K), leucine (L), methionine (M), asparagine (N), proline (P), glutamine (Q), arginine (R), serine (S), threonine (T), valine (V), tryptophan (W), and tyrosine (Y).

The term "peptoid" as used herein refers to a non-peptide monomer of the general formula $(R)_n$-X-$(L)_m$, where R is a side chain group, n is at least 1, L is a linking group, m is at least 2, and X is a small organic radical. It is preferred to select L radicals that may be individually protected and deprotected. Preferably n will be 1 or 2 and m will be 2. Monomers wherein m is 3 or greater may be used to form branched active polymers. Presently preferred monomers are N-substituted glycine derivatives of the formula



wherein R is alkyl of 2-6 carbon atoms, haloalkyl of 1-6 carbon atoms wherein halo is F, Cl, Br, or I, alkenyl of 2-6 carbon atoms, alkynyl of 2-6 carbon atoms, cyclolkyl of 3-8 carbon atoms, alkoxyalkyl of 2-8 carbon atoms, aryl of 6-10 carbon atoms, arylalkyl of 7-12 carbon atoms, arylalkyl of 7-12 carbon atoms substituted with 1-3 radicals independently selected from halo and nitro and hydroxy, aminoalkyl of 1-6 carbon atoms, hydroxyalkyl of 1-6 carbon atoms,

carboxy, carboxyalkyl of 2-6 carbon atoms, carboalkoxy-alkyl of 3-10 carbon atoms, carbamyl, carbamylalkyl of 2-6 carbon atoms, imidazolyl, imidazolylalkyl of 4-10 carbon atoms, pyridyl, pyridylalkyl of 6-10 carbon atoms, piperidyl, piperidylalkyl of 5-10 carbon atoms, indolyl, or indolyalkyl of 9-15 carbon atoms. Thus, active polymers composed of these monomers are equivalent to polyglycine having side chains attached at each nitrogen. These and other monomers are described in copending application USSN 07/715,823, incorporated herein by reference, and PCT WO91/19735.

The terms "coding" and "encoding" indicated that one or more coding monomers corresponds directly and uniquely to a given active monomer, e.g., conventional nucleic acids encode (in groups of three) the 20 natural amino acids. The number of coding monomers used for each code depends on the number of different coding monomers and the number of different active monomers. Typically, the number of different active monomers used will range from about 5 to about 30. A basis set of 4 coding monomers can encode up to 16 active monomers taken in "codons" of 2 coding monomers. By increasing the coding monomer basis set to five distinct monomers, one can encode up to 25 different peptide/peptoid monomers. A basis set of 4 coding monomers can encode up to 64 peptide/peptoid monomers taken in "codons" of 3 coding monomers. Note that one can make the code degenerate or nondegenerate, and can insert additional coding information into the sequence. For example, one may wish to begin each codon with the same base (e.g., G), using that base only in the first position, thus unambiguously identifying the beginning of each codon. As a practical matter, the group of monomers selected for use as coding monomers will form polymers that are easier to

sequence than the active polymers, i.e., the coding monomers may be more readily identified using present day sequencing technology as compared to the monomer of the active polymers. With current technology, the order of preference for coding monomers is nucleic acids > peptides > peptoids. Nucleic acids have the additional advantage that the coding sequence may be amplified by cloning or PCR (polymerase chain reaction) methods known in the art.

The term "active polymer" and/or "binding polymer" refers to a polymer having a desired biological activity. Suitable biological activities include binding to natural receptors, pharmaceutical effects, immunogenicity/antigenicity, and the like. "Immunogenicity" refers to the ability to stimulate an immune response (whole or partial serum-mediated immunity and/or cell-mediated immunity) in a bird or mammal following administration. Antigenicity requires only that the active polymer bind to the antigen-binding site of an antibody. Pharmaceutical activities, for the purposes of this invention, will generally depend on the ability of the active polymer to bind a protein, carbohydrate, lipid, nucleic acid, or other compound present in the subject. For example, an active polymer may bind to a cell surface receptor and compete with the receptor's natural ligand, with or without activation of the receptor. Other useful pharmaceutical activities include cleavage of endogenous molecules (e.g., protease activity, nuclease activity, and the like), catalysis of reactions either primarily or as a cofactor, donation of functional groups (e.g., acyl, ATP, alkyl, and the like), pore formation, and the like. Active polymers comprise a series of monomers which are linked sequentially. The monomers will generally be peptides, peptoids, or carbohydrates in the practice of the instant invention.

The term "mixture" as used herein refers to a composition having a plurality of similar components in a single vessel.

The term "couple" as used herein refers to formation of a covalent bond.

The term "coupling moiety" refers to a soluble or insoluble support to which can be attached one or more active monomers and the corresponding encoding monomers. Insoluble supports ("solid support means") may be any solid or semi-solid surface which is stable to the reaction conditions required for synthesis of the active and coding polymers, and is suitable for covalently attaching and immobilizing both polymers, for example, most resins commonly employed in DNA and peptide synthesis, such as MBHA, Rink, and the like. The particular resin used will depend upon the choice of coding and active polymers and their associated synthetic chemistries. Soluble coupling moieties are molecules having functional groups to which active and coding monomers may be attached. Each soluble coupling moiety must be able to accommodate at least one coding polymer and at least one active polymer, although the active and coding polymers need not be present in a 1:1 ratio. The soluble coupling moiety may be as simple as an amino acid having an functional group in its side chain, or may be as complex as a functionalized (soluble) polymer.

The term "conjugate" as used herein refers to the combination of any "active polymer" and its associated "coding" polymer. The conjugate may be formed using a "coupling moiety" or by binding both the "active polymer" and "encoding polymer" to the same support surface in close proximity with each other so that the two polymers are "associated" with each other. When both polymers are bound to the same support surface, such as a small bead, the encoding polymer can be readily sequenced off of the bead and

the other "active polymers" remaining on the bead will be identified once the encoding sequence is known.

B. Related Libraries and Synthesis Methodologies for producing same.

There are many limitations with the current technologies for probing the receptor-binding properties of peptide libraries. Filamentous bacteriophage libraries offer the largest source of peptide diversity ($\approx 10^7$-10^8 different components) of any current technology to date (Scott, J. & Smith, G., Science, (1990), 249, 386-390; Devlin, J., Panganiban, L. & Devlin, P., Science, (1990), 249, 404-406; Cwirla, S., Peters, E., Barret, R. & Dower, W., Proc. Natl. Acad. Sci. U.S.A., (1990), 87, 6378-6382). These libraries, however, are limited to the natural set amino acids, suffer from biological biases (i.e., varying rates of growth, proteolysis, etc.) and also suffer, in practice, from high levels of background binding. The present invention is designed to overcome these difficulties.

Multiple-peptide synthesis technology has substantially increased the ability to generate individual peptides (Geysen, H., Meloen, R. & Barteling, S., Proc. Natl. Acad. Sci. U.S.A., (1984), 81, 3998-4002; Houghten, R., Proc. Natl. Acad. Sci. U.S.A., (1984), 5131-5135; Schnorrenberg, G. & Gerhardt, H., Tetrahedron, (1989), 45, 7759-7764; Gausepohl, H., Kraft, M., Boulin, C. & Frank, R. in Peptides: Chemistry, Structure and Biology (Proceedings of the 11th American Peptide Symposium, (1990), eds. Rivier J. & Marshall, G., (ESCOM, Leiden), pp. 1003-1004; Frank, R. & Döring, R., Tetrahedron, (1988), 44, 6031-6040; Fodor, S., Read, J., Pirrung, M., Stryer, L., Lu, A. & Solas, D., Science, (1991), 251, 767-773). The synthesis of $\approx 10^4$ individual peptides per cm^2 of glass wafer represents the diversity limit of this technology (Fodor, S.,

Read, J., Pirrung, M., Stryer, L., Lu, A. & Solás, D., Science, (1991), 251, 767-773). A mixed-resin algorithm method (Furka, A., Sebestyén, M., Asgedom M. & Dibó, G., Int. J. Peptide Protein Res., (1991), 37, 487-493) has recently been used to generate solution-phase libraries (Houghten, R., Pinilla, C., Blondelle, S., Appel, J., Dooley, C. & Cuervo, J., Nature, (1991), 354, 84-86) and resin-bound peptide libraries (Lam, K., Salmon, S., Hersh, E., Hruby, V., Kazmiersky, W. & Knapp, R., Nature, (1991), 354, 82-84) that contain ≈10^6 and ≈10^7 components, respectively. The solution-phase libraries offer the advantage of providing quantitative receptor-binding information (Zuckermann, R., Kerr, J., Siani, M., Banville, S. & Santi, D.V., Proc. Natl. Acad. Sci. U.S.A., 89, 4505-4509 (1992)). Furthermore, these libraries allow the affinity of the solution conformation of a ligand to be determined, a quantity that is essential for rational drug design. An apparatus for the automated synthesis of equimolar peptide mixtures is disclosed in Zuckermann, R.N., Kerr, J.M., Siani, M.A. & Banville, S.C., Int. J. Pep. Pro. Res., (1992), 40, 498-507.

The publications cited and discussed above can be used in producing the active or binding polymer which is used in producing the conjugate of the present invention. Accordingly, the disclosures of all of these publications are incorporated herein by reference in order to disclose peptide and peptoid synthesis methodology. Although the methodology discussed within these references is extremely valuable with respect to the production of large amounts of different types of binding polymers, the mixtures of polymers produced by this methodology are often so large and complex that there are many practical limitations with respect to their actual analysis and use. The present invention can be readily applied with such synthesis methodologies in

order to provide an efficient, commercially practical method of analyzing the proteins produced using such methodology.

Both the mixed-resin and solution-phase methods, however, do not allow incorporation of many non-standard amino acids because of the limitations of peptide analysis. Resin-bound peptide libraries, in particular, suffer from a relatively slow rate of analysis (peptide sequencing at 3 beads per day) and are limited in complexity to $\approx 10^7$ beads/ml. In order to generate a "complete" peptide library, there must be multiple copies (>10) of any given peptide sequence. This becomes problematic at the sequencing stage because the same "hit" sequence may appear multiple times. The alternative is to work with libraries that are not complete at the risk of losing sequences that bind.

When using the methodology of the present invention, the sequence of a biologically active protein can be determined even without isolating the protein of interest. This can be done by synthesizing large numbers of different proteins on large numbers of different support surfaces such as small beads. An encoding polymer is attached to beads to identify each protein. A sample to be tested is then brought into contact with the beads and the beads are observed with respect to which proteins bind to a receptor site in the sample. The bead having the receptor bound thereon is analyzed by sequencing the coding polymer which has also been synthesized on the bead. When the encoding polymer has been sequenced, the sequence of the active polymer, which may be a peptide, can be readily deduced. Thus, the present invention makes it possible to determine the activity and sequence an active polymer, such as a biologically active peptide, without ever isolating the peptide.

C. General Methodology

This invention describes a methodology for the synthesis and screening of large synthetic polymer libraries that contain non-standard amino acids and even non-amide based polymers. The strategy utilizes a modified mixed-resin peptide synthesis methodology to simultaneously synthesize two polymer sequences: one polymer strand (the "binding" strand) is synthesized for the intended purpose of receptor binding, and the second strand (the "coding" strand) contains standard amino acids or deoxyribonucleotides that encodes for the binding strand (Figure 1). The ability to decipher the binding sequence by analysis of the coding strand with standard peptide or oligonucleotide techniques allow the inclusion of a wide variety of novel building blocks and conformational constraints into a diverse ligand library.

This invention also describes a methodology to increase the size (>10^8) and screening rate of a ligand library. The method uses two polymers as above, but specifically utilizes an oligodeoxyribonucleotide for the "coding" strand. The use of DNA as the coding strand allows for an increased sensitivity of detection (fmol vs pmol for peptide analysis). This increased sensitivity allows for a larger library size since the amount of polymer needed for detection is reduced dramatically. The rate of sequence determination of receptor binders is increased since many samples can be analyzed in parallel.

In order to couple a polymer's sequence information with a peptide or oligonucleotide sequence, there needs to be a method that unambiguously correlates each polymer to each other. Thus, when any particular non-standard amino acid (or other monomer) is added to a "binding" polymer chain, the corresponding information (amino acid or

nucleotide monomer) must also be added to the "coding" strand. A "genetic code" is thus established (Table 1) where each binding monomer corresponds to (a multiple) of standard amino acids or nucleotides on the coding strand. For example, the use of three standard amino acids or nucleotides, in a 3:1 ratio with a novel monomer, would allow for the unambiguous representation of 27 novel monomers.

TABLE 1. Custom genetic code.

# of bases	"codon" length	# of monomers coded for
2	3	8
2	4	16
2	5	32
3	2	9
3	3	27
4	2	16
4	3	64

The synthesis of coded libraries requires a modified mixed-resin algorithm (Figure 2). The resin beads are divided into equal portions, a unique monomer is added to the "binding" strand, followed by the coupling of a corresponding amino acid or nucleotide to the "coding" strand. The resin aliquots are then combined to generate a mixture. A set of compatible protecting groups is thus required to preferentially deprotect and extend each strand independently.

Two synthesis formats are possible for amptide libraries, one that generates resin-bound libraries and one that generates solution-phase libraries (Figure 3). Resin-bound libraries can be synthesized using non-hydrolyzable linkers that are derivatized with the "binding" and "coding" monomers strands. Solution-phase libraries can be synthesized

as a 1:1 polymer:peptide/DNA conjugate via a hydrolyzable linker attached to the resin.

Peptide as the "Coding" strand

The use of base-labile Fmoc-protected monomers and acid-labile (N^{α}-Ddz-protected amino acids (Birr, C., Nassal, M., Pipkorn, R., Int. J. Peptide Protein Res., (1979), 13, 287-295), for example, allow for selective deprotection and coupling to two individual polymer strands. Resin-bound libraries can be generated by the derivatization of non-hydrolyzable resins with a 1:1 ratio (or any desired ratio) of Fmoc:Ddz monomers (Figure 4). This introduces two differently protected amino acids that an be extended independently. Solution-phase libraries that contain a 1:1 ratio of binding:coding strands can be synthesized by using a hydrolyzable Fmoc-Lysine(Moz)-OH linker that allowed for chain growth at both the α- and ε-amino groups. Amino acids which do not contain functional groups are preferred for the "coding" strand in order to minimize unwanted binding interactions.

The receptor-binding ligand can be identified by bead staining techniques (Lam, K., Salmon, S., Hersh, E., Hruby, V., Kazmiersky, W. & Knapp, R., Nature, (1991), 354, 82-84) and the sequence determined by N-terminal Edman degradation. In order to ensure that only the "coding" strand is sequenced, it is essential that the N-terminus of the "binding" strand be acetylated or otherwise made non-sequencable.

DNA as the "Coding" Strand

The construction of libraries with DNA as the coding strand is similar to those with peptides but offers several advantages: the information storage and replicative properties of DNA allow for

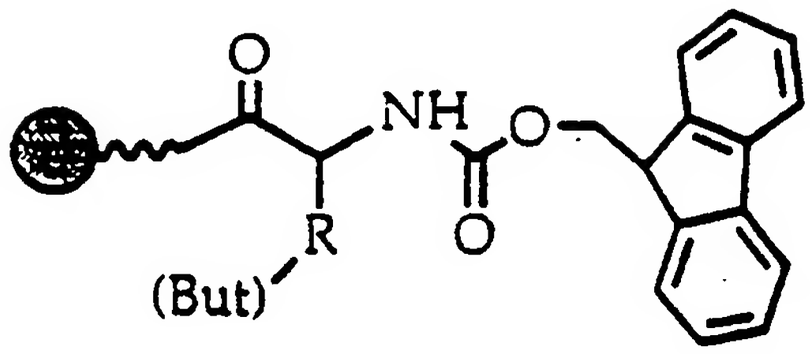
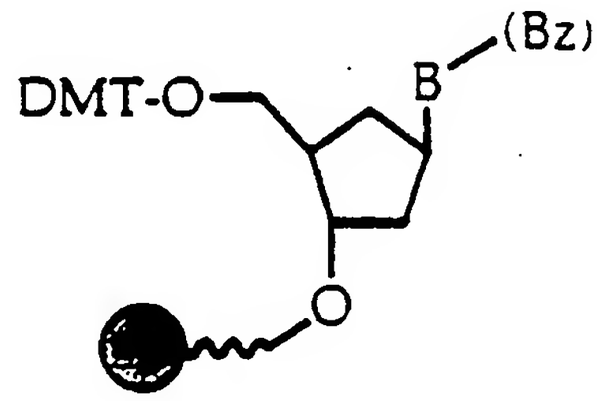
increased sensitivity of detection, a larger library size and an increased rate of sequence determination.

The synthesis of DNA as the coding polymer requires compatibility between the assembly of Fmoc-based monomers and standard DNA chemistry. These synthesis strategies are likely to be compatible ((a) Juby, C., Richardson, C. & Brousseau, R., Tet. Letters, (1991), 32, 879-882. (b) Haralambidis, J., Duncan, L., Angus, B. & Tregear W., Nucleic Acid Res., (1990), 18, 493-499) (see Table 2). Alternatively, allyl-based protection strategies exists for both peptide (Lyttle, M.H.; Hudson, D., Peptides: Chemistry and Biology (Proceedings of the 12th American Peptide Symposium): Smith, J. and Rivier, J.E., Eds.; ESCOM, Leiden, 1992, pp. 583-584) and oligodeoxyribonucleotide (Hayakawa, Y., Wakabayashi, S., Kato, H. & Noyori, R., J. Am. Chem. Soc., (1990), 112, 1691-1696) synthesis. The assay of solution-phase libraries can be facilitated by using only pyrimidines in the coding strand, thereby avoiding the potential problem of base pairing between individual strands.

Strategies for the synthesis of coding and active polymers, as well as matching the active polymer with a coding sequence to provide a genetic tag are described in Brenner et al. Proc. Natl. Acad. Sci. U.S.A., 89:5381-5383 (June 1992) which is incorporated herein by reference.

TABLE 2

Compatibility of DNA vs. Peptide Synthesis Chemistry

Peptide chemistry	Oligonucleotide Chemistry
	

Permanent Protecting Groups:

-OH	t-butyl ether	A,C	benzoyl amide
$-CO_2H$	t-butyl ester	G	isobutyryl
$-NH_2$	t-boc	-P=O	cyanoethyl, methyl
his, cys	trityl		
arg	sulfonyl		

removed by:

85% trifluoroacetic acid — conc. NH_4OH 55°C 5 hours
2 hours @ room temp

Temporary protecting groups:

9-fluorenylmethoxycarbonyl — 4,4-dimethoxytrityl

removed by:

20% piperidine — 3% trichloroacetic acid

Cleavage from solid support:

85% trifluoroacetic acid — conc. NH_4OH 55°C 5 hours
2 hours @ room temp

TABLE 2 (Cont)

Compatibility of DNA vs. Peptide Synthesis Chemistry

Std. conditions	Alternative	Problem addressed
t-Butyl carbamate/ ester	allyl	amino acid deprotection from detritylation reagent
20% Piperidine	2% DBU	substitution at exocyclic amine
cyanoethyl phosphate	allyl	acylation of phosphate
NH_4OH	ethylenediamine/ EtOH	racemization
iodine	t-Butylhydro-peroxide	Tyr, Met, Cys oxidation
controlled pore glass	polystyrene resin	resin transferring
T, C & G	T, C, G & A	depurination
T, C	T, C, G & A	base pairing

Resin-bound libraries can be synthesized by using non-hydrolyzable linkers to attach both the C-terminus of the peptide and the 3'-end of the oligonucleotide to the same bead. Solution-phase libraries can be synthesized as a 1:1 peptide-oligonucleotide conjugate, in which the C-terminus of the peptide is attached to the 3'-end of the oligonucleotide through a hydrolyzable Fmoc-Ser(O-Dmt) linker which is attached to the resin.

The identification of binders in the resin-bound peptide libraries can be detected by the bead staining methodology (Lam, K., Salmon, S., Hersh, E., Hruby, V., Kazmiersky, W. & Knapp, R., Nature, (1991), 354, 82-84). Although the peptides are bound to a solid-phase, there does not have to be a 1:1 peptide-oligonucleotide ratio since the DNA can be amplified prior to the determination of its sequence. In fact, less DNA is preferred so that there will be less interference with the polymer's binding properties. Once a bead is identified, the DNA sequence is determined (Stahl, S., Hultman, T., Olsson, A., Mois T., et al., Nucleic Acid Res., (1988), 16, 3025-3038) after PCR amplification or by thermal-cycle sequencing (Figure 8). This requires the inclusion of one or more primer sites neighboring the coding region of the oligonucleotide. Similarly, the use of solution-phase libraries requires isolation of each sequence from each other. This can be accomplished by restricting the DNA after PCR amplification and inserting it into M13 (or other suitable vector) for clonal isolation and sequencing (Figure 9).

EXAMPLES

The following examples will provide those skilled in the art with a complete disclosure of how to make and use the invention and are not intended to limit the scope of the invention. Efforts have been made to insure accuracy with respect to numbers used

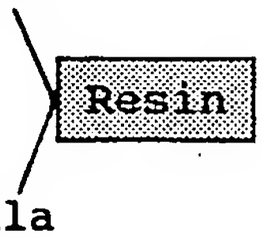
(e.g. amounts, temperature, etc.), but some experimental error and deviation should be accounted for. Unless indicated otherwise, parts or parts by weight, molecular weight is weight average molecular weight, temperature is in degrees centigrade and pressure is at or near atmospheric.

Example 1

The independent synthesis of two unambiguously correlated sequences has been successfully completed. The subsequent sequence analysis of the "coding" strand has also been demonstrated. For convenience, two peptide sequences were chosen. The "binding" strand was synthesized with N^{α}-Fmoc-protected amino acids and the "coding" strand was synthesized with N^{α}-Ddz-protected amino acids. The simultaneous synthesis of the two peptide strands was tested in two formats, 1) resin-bound peptide library synthesis and 2) solution-phase peptide libraries using a hydrolyzable Fmoc-Lys(Moz)-OH linker (Wang, S.S.; Chen, S.T, Wang, K.T., and Merrifield, R.B., Int. J. Peptide Protein Res., (1987), 30, 662-667). These syntheses were performed on single peptides (not libraries) as a demonstration of research concept and in order to allow the full characterization of the synthesis products.

A. Synthesis of a Resin-Bound Library Model

"Binding" Sequence: Ac-Arg-Leu-Val-Thr-His
(Fmoc peptide)



"Coding" Sequence: H_2N-Ala-Ser-Gly-Glu-Phe-Ala
(Ddz peptide)

Synthesis Scheme:

Step #	Description
1	Derivatization of MBHA resin with 1:1-Ddz-Ala-OH: Fmoc-His(Trt)-OH
2	Deprotection of "binding" strand with 20% piperidine/DMF
3	Coupling of second "binding" amino acid, Fmoc-Thr(But)-OH
4	Deprotection of "coding strand" with 7.5% TFA/CH_2Cl_2
5	Coupling of second "coding" amino acid, Ddz-Phe-OH
6	Repeat steps 2-5 with alternative Fmoc and Ddz deprotection and corresponding amino acid coupling
7	Final Deprotection of Fmoc followed by acetylation
8	TFA deprotection of side-chain groups

After the TFA deprotection, the model library bead has two independently synthesized sequences and is ready for assay. Only the coding strand has a free α-amino group and can be characterized by N-terminal Edman degradation. The binding strand is acetylated and there-fore will not interfere with the sequencing. The two peptides were cleaved from the resin with HF thereby providing both the "binding" and "coding" sequences as free peptides. The amino acid composition, mass spectro-scopy and N-terminal sequencing data are consistent with the correct products. (See Figures 5, 6 and 7.)

Mass Spectrometry:

	theoretical	observed
Ddz peptide	580.2	580.2
Fmoc peptide	666.9	666.4

Amino Acid Composition:

Fmoc Peptide			Ddz Peptide		
	Theor. (%)	Observ. (%)		Theor. (%)	Observ. (%)
His	20	21	Ala	33	33
Thr	20	20	Glu	17	17
Val	20	19	Phe	17	17
Leu	20	29	Gly	17	15
Arg	20	20	Ser	17	15

N-Terminal Edman Sequencing of Resin beads (coding peptide only):

Cycle #	amino acid	pmol
1	Ala	45
2	Ser	12
3	Gly	30
4	Glu	28
5	Phe	28
6	Ala	29

Example 2

Synthesis of a Solution-Phase Peptide Library Model

In this example, a 1:1 solution-phase adduct between a "binding" and a "coding" strand was synthesized and fully characterized. The "binding" strand was assembled with Fmoc-protected monomers, and the "coding" strand was assembled with Ddz-protected monomers.

"Binding" sequence: Ac-Glu-Ser-Thr-Arg-Pro-nLeu-Lys-ß-Ala-NH_2
(Fmoc peptide)

"Coding" sequence: H_2N-Gly-Ala-Phe-Gly-Ala-Phe-CONH
(Ddz peptide)

Synthesis Scheme:

Step #	Description
1	Derivatization of Rink Resin with Fmoc-B-Ala-OH spacer
2	Fmoc deprotection with 20% piperidine/DMF
3	Coupling of Fmoc-Lys (4-methoxybenzyloxycarbonyl)-OH
4	Fmoc deprotection of "binding" strand with 20% piperidine/DMF
5	Coupling of first "binding" monomer Fmoc-nLeu-OH
6	Cleavage of "coding" strand 4-Methoxybenzyloxycarbonyl (Moz) group with 7.5% TFA/CH_2Cl_2
7	Coupling of first "coding" amino acid Ddz-Phe-OH
8	Repeat steps 4-7 with corresponding amino acids
9	Final Fmoc deprotection and acetylation
10	Cleavage and deprotection of resin sample with TFA

Following TFA cleavage and deprotection, the model solution-phase library contains a 1:1 Fmoc/Ddz conjugate peptide. One peptide sequence was synthesized and not a mixture in order to fully characterize the reaction product. The amino acid composition and mass spectroscopy data are consistent with the correct product. In addition, the "binding" and "coding" hybrid peptides were tested in a competition ELISA format. The ELSTRPnL "binding" sequence binds to an anti-gp120 antibody with submicromolar affinity. This value was not affected by the presence of the "coding" peptide.

Mass Spectroscopy:

	Theoretical	Observed
Fmoc/Ddz peptide conjugate:	1492.7	1492.6

Amino Acid Composition:

Fmoc Peptide			Ddz Peptide		
	Theor. (%)	Observ. (%)		Theor. (%)	Observ. (%)
Glu	7.1	7.0	Phe	4.2	14.7
Ser	7.1	7.1	Ala	14.2	14.4
Thr	7.1	7.2	Gly	14.2	13.8
Arg	7.1	7.2			
Pro	7.1	7.9			
Nleu	7.1	6.3			

The instant invention is shown and described herein in what is considered to be the most practical, and preferred embodiments. It is recognized, however, that departures may be made therefrom which are within the scope of the invention, and that obvious modifications will occur to one skilled in the art upon reading this disclosure.

CLAIMS

1. An assay conjugate, comprising:

an active polymer comprising monomers selected from the group consisting of peptide and peptoid monomers;

an encoding polymer comprising encoding monomers, wherein the encoding polymer corresponds to and allows identification of the active polymer; and

a coupling moiety covalently coupled to the active peptide and the encoding polymer.

2. The conjugate of claim 1, wherein the coupling moiety comprises a solid support.

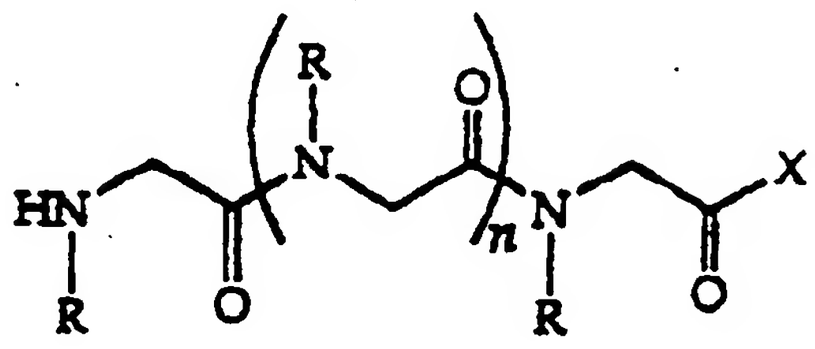
3. The conjugate of claim 1, wherein the coupling moiety comprises a soluble linking group.

4. The conjugate of claim 1, wherein all active polymers coupled to a single selected solid support are identical.

5. The conjugate of claim 1, wherein the active polymer comprises a polypeptide and the encoding polymer comprises a DNA or RNA oligonucleotide.

6. The conjugate of claim 1, wherein the active polymer comprises a polypeptoid and the encoding polymer comprises a DNA or RNA oligonucleotide.

7. The conjugate of claim 6, wherein the polypeptoid comprises a polymer of monomers of the formula:



wherein R is alkyl of 2-6 carbon atoms, haloalkyl of 1-6 carbon atoms wherein halo is F, Cl, Br, or I, alkenyl of 2-6 carbon atoms, alkynyl of 2-6 carbon atoms, cyclolkyl of 3-8 carbon atoms, alkoxyalkyl of 2-8 carbon atoms, aryl of 6-10 carbon atoms, arylalkyl of 7-12 carbon atoms, arylalkyl of 7-12 carbon atoms substituted with 1-3 radicals independently selected from halo and nitro and hydroxy, aminoalkyl of 1-6 carbon atoms, hydroxyalkyl of 1-6 carbon atoms, carboxy, carboxyalkyl of 2-6 carbon atoms, carboalkoxy-alkyl of 3-10 carbon atoms, carbamyl, carbamylalkyl of 2-6 carbon atoms, imidazolyl, imidazolylalkyl of 4-10 carbon atoms, pyridyl, pyridylalkyl of 6-10 carbon atoms, piperidyl, piperidylalkyl of 5-10 carbon atoms, indolyl, or indolyalkyl of 9-15 carbon atoms.

8. A mixture of conjugates of claim 1, wherein the mixture comprises at least two distinct active polymers, the coupling moiety is a solid support, distinct active polymers are covalently coupled to separate solid supports and a distinct encoding polymer corresponding to each active polymer is covalently coupled to the support coupled to its corresponding active polymer.

9. The mixture of claim 8, wherein the mixture comprises at least ten distinct active polymers.

10. The mixture of claim 9, wherein the mixture comprises at least 100 distinct active polymers.

11. A conjugate, comprising:

a biologically active peptide comprised of five or more amino acids;

an encoding polymer comprised of nucleic acids wherein one or more nucleic acids within the encoding polymer correspond to and can be readily identified with the amino acids of the active polymer; and

a coupling moiety covalently coupled to the active peptide and the encoding polymer.

12. The conjugate of claim 11, wherein the amino acids are selected from the group consisting of alanine, cysteine, aspartic acid, glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, leucine, methionine, asparagine, proline, glutamine, arginine, serine, threonine, valine, tryptophan, and tyrosine.

13. The conjugate of claim 11, wherein the encoding polymer includes three nucleotides for each amino acid of the active polymer and the three nucleotides are nucleotides which naturally encode the corresponding amino acid of the active polymer.

14. The conjugate of claim 11, wherein the coupling moiety comprises a solid support.

15. The conjugate of claim 14, wherein the solid support is in the form of a spherical bead having a diameter of less than one centimeter.

16. The conjugate of claim 11, wherein the coupling moiety comprises a soluble linking group.

17. A method of synthesizing an encoded peptide or peptoid polymer, the method comprising:

a) providing a coupling moiety;

b) coupling to the coupling moiety an active monomer, and coupling to the coupling moiety an encoding monomer which corresponds to the active monomer to form a conjugate having a bound active monomer and a bound encoding monomer; and

c) repeating step b) until an active polymer of the desired length is obtained.

18. The method of claim 17 wherein the active monomers are amino acids and the encoding monomers are nucleic acid bases.

19. A method of synthesizing a mixture of encoded polymers, the method comprising:

a) providing a plurality of coupling moieties;

b) dividing the plurality of coupling moieties into a plurality of aliquots;

c) within each aliquot, coupling to each coupling moiety an active monomer, and coupling to each said coupling moiety an encoding monomer which corresponds to the active monomer to form a plurality of conjugates having a bound active monomer and a bound encoding

monomer, each in a separate aliquot, wherein different aliquots may contain different active monomers;

d) combining the aliquots of conjugates to form a mixture of conjugates; and

e) repeating steps b-d) until a mixture of active polymers of the desired length is obtained.

20. The method of claim 19, wherein the active monomers comprise activated amino acids and the coupling moiety comprises a solid support.

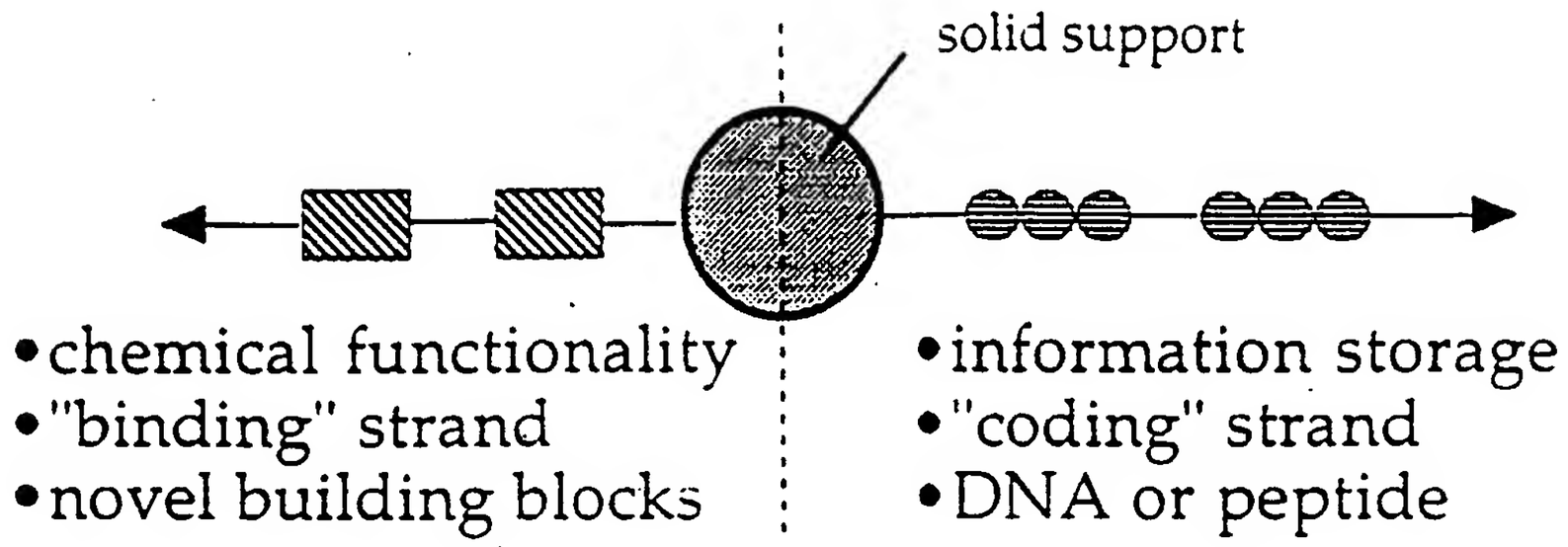

solid support
•chemical functionality
•"binding" strand
•novel building blocks
•information storage
•"coding" strand
•DNA or peptide

FIGURE 1

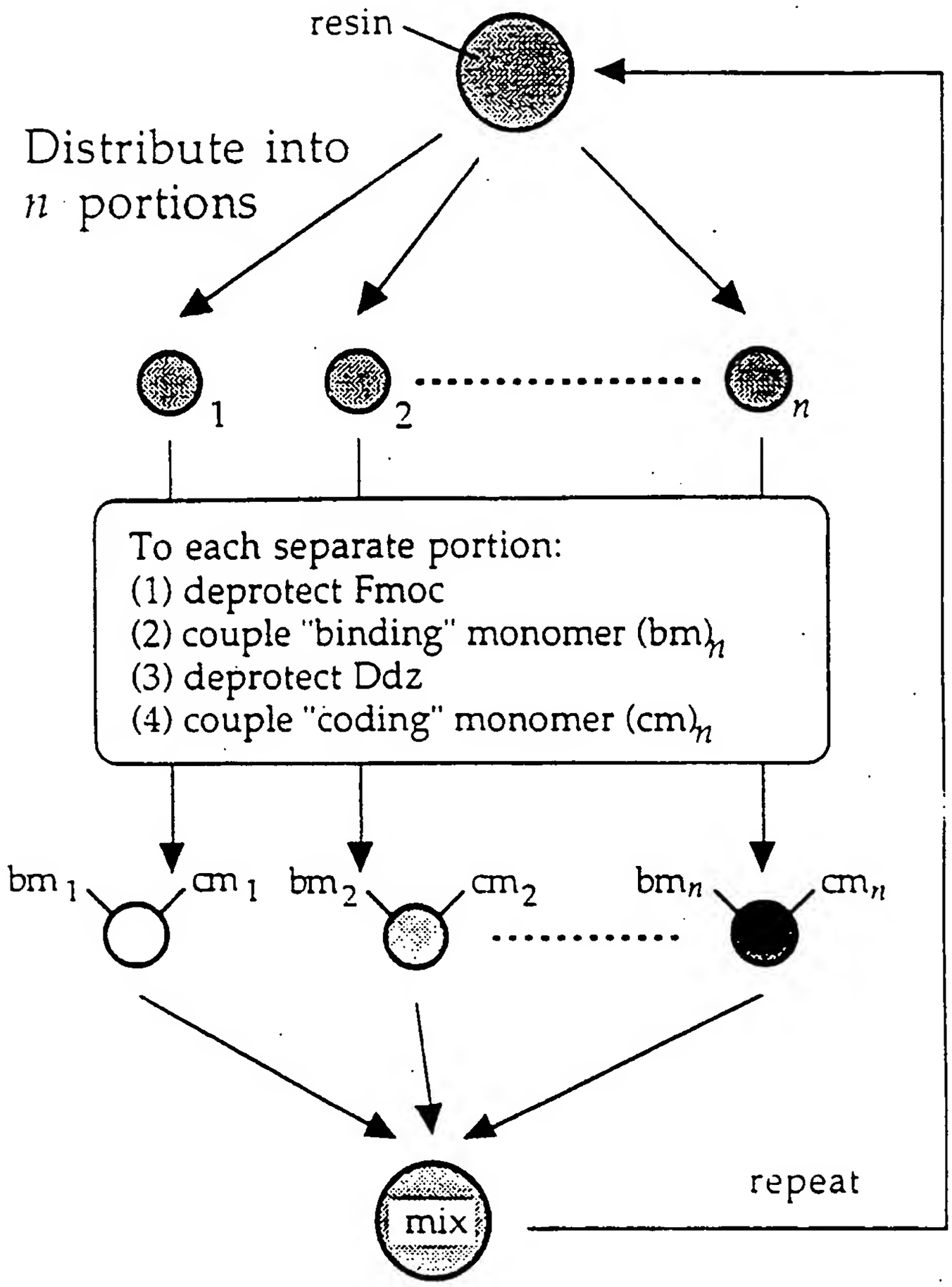
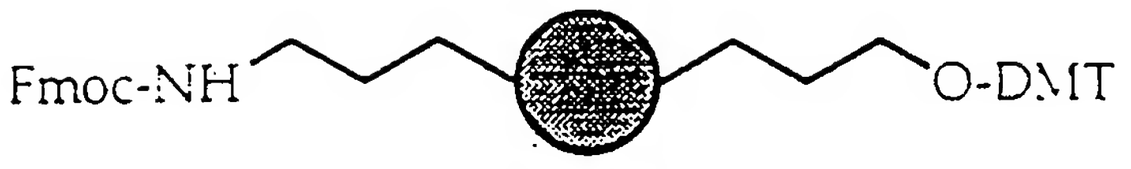

resin
Distribute into
n portions
1
2
n
To each separate portion:
(1) deprotect Fmoc
(2) couple "binding" monomer (bm)n
(3) deprotect Ddz
(4) couple "coding" monomer (cm)n
bm1
cm1
bm2
cm2
bmn
cmn
mix
repeat

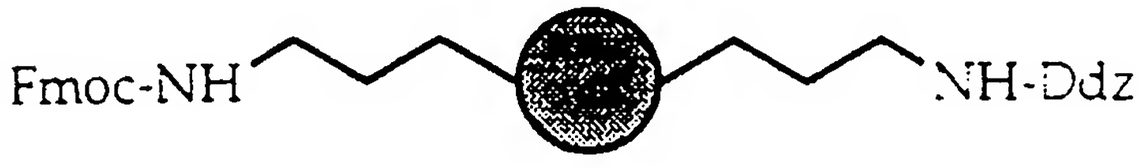
FIGURE 2

(1) Resin-bound:

polymer/DNA

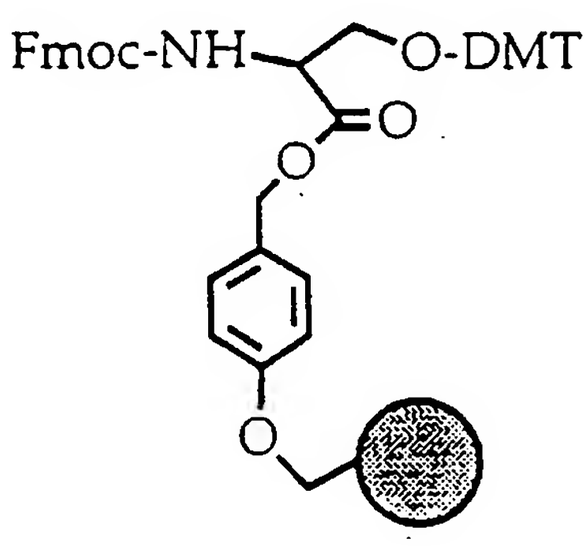


polymer/peptide



(2) Solution-phase conjugate:

polymer/DNA



polymer/peptide

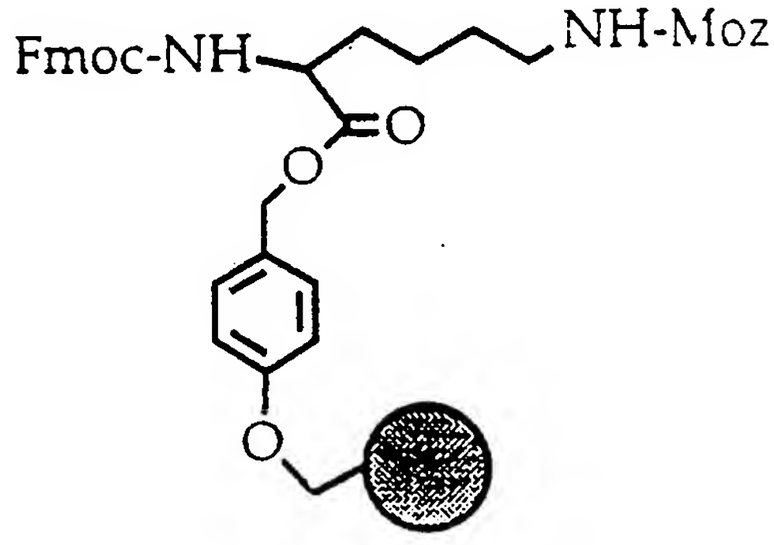


FIGURE 3

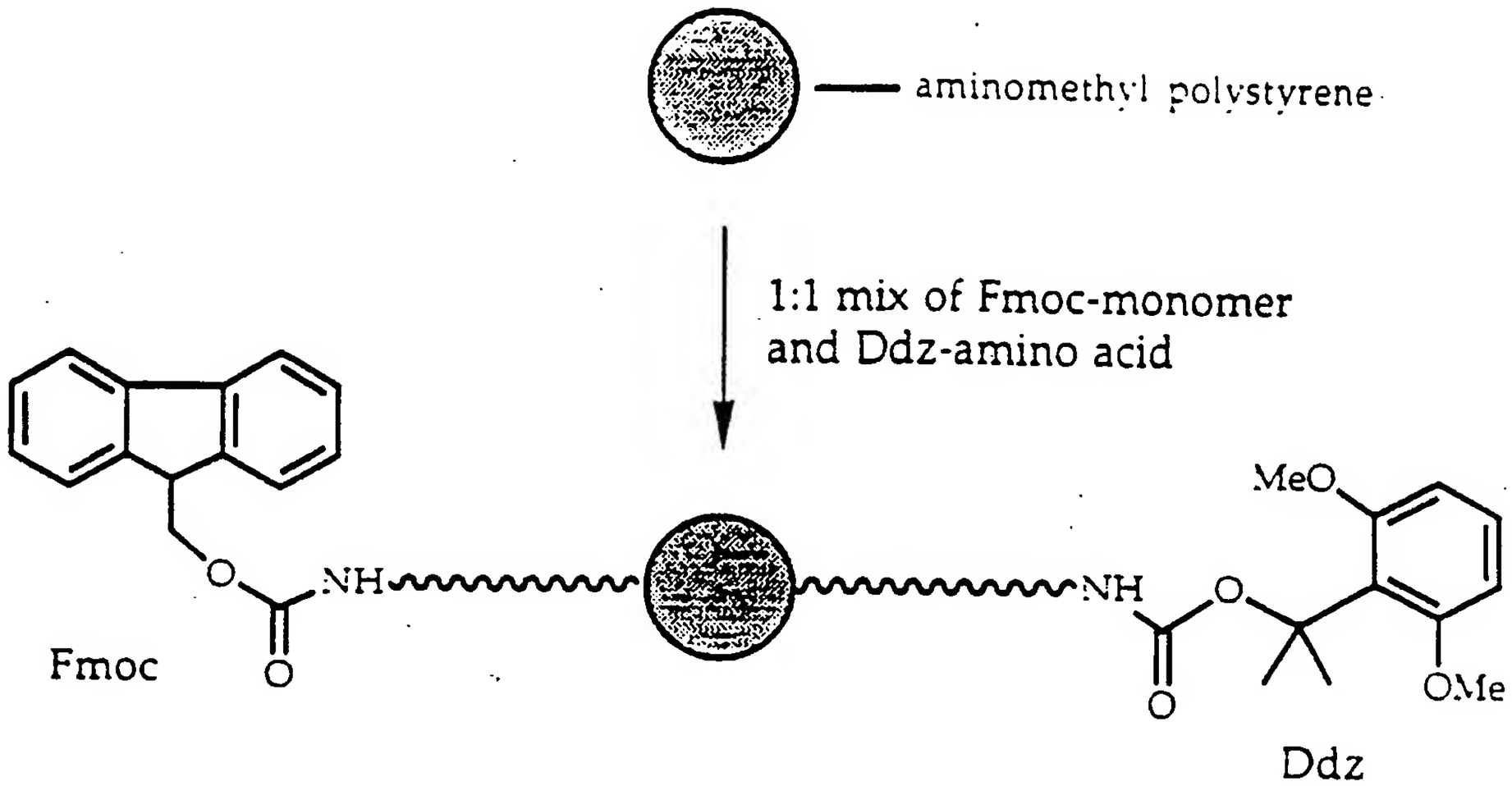

aminomethyl polystyrene
1:1 mix of Fmoc-monomer
and Ddz-amino acid
Fmoc
NH
O
NH
O
MeO
OMe
Ddz

FIGURE 4

HPLC chromatogram of HF cleaved/deprotected resin sample:

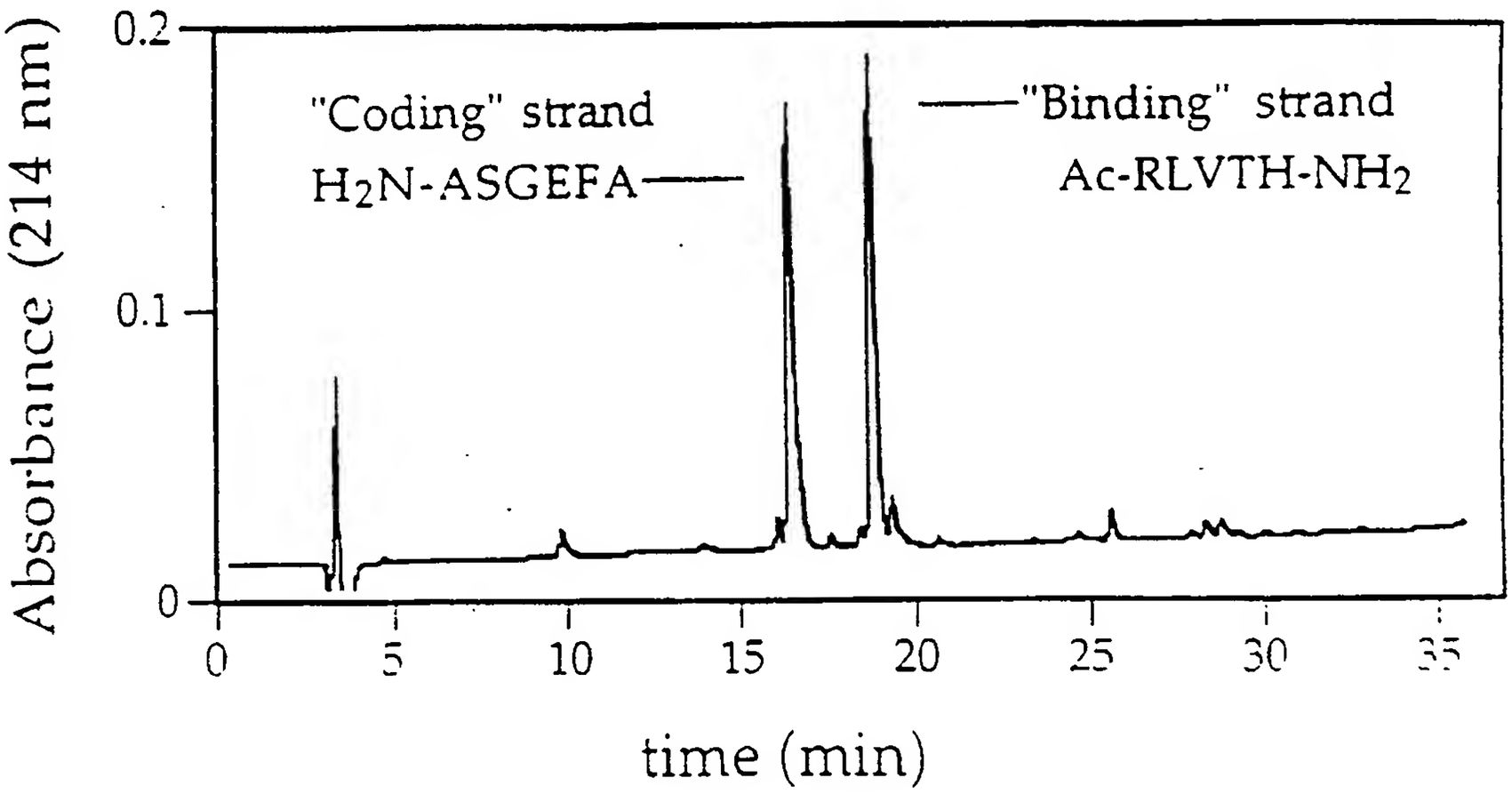


FIGURE 5

HPLC chromatogram of TFA cleaved / deprotected peptide:

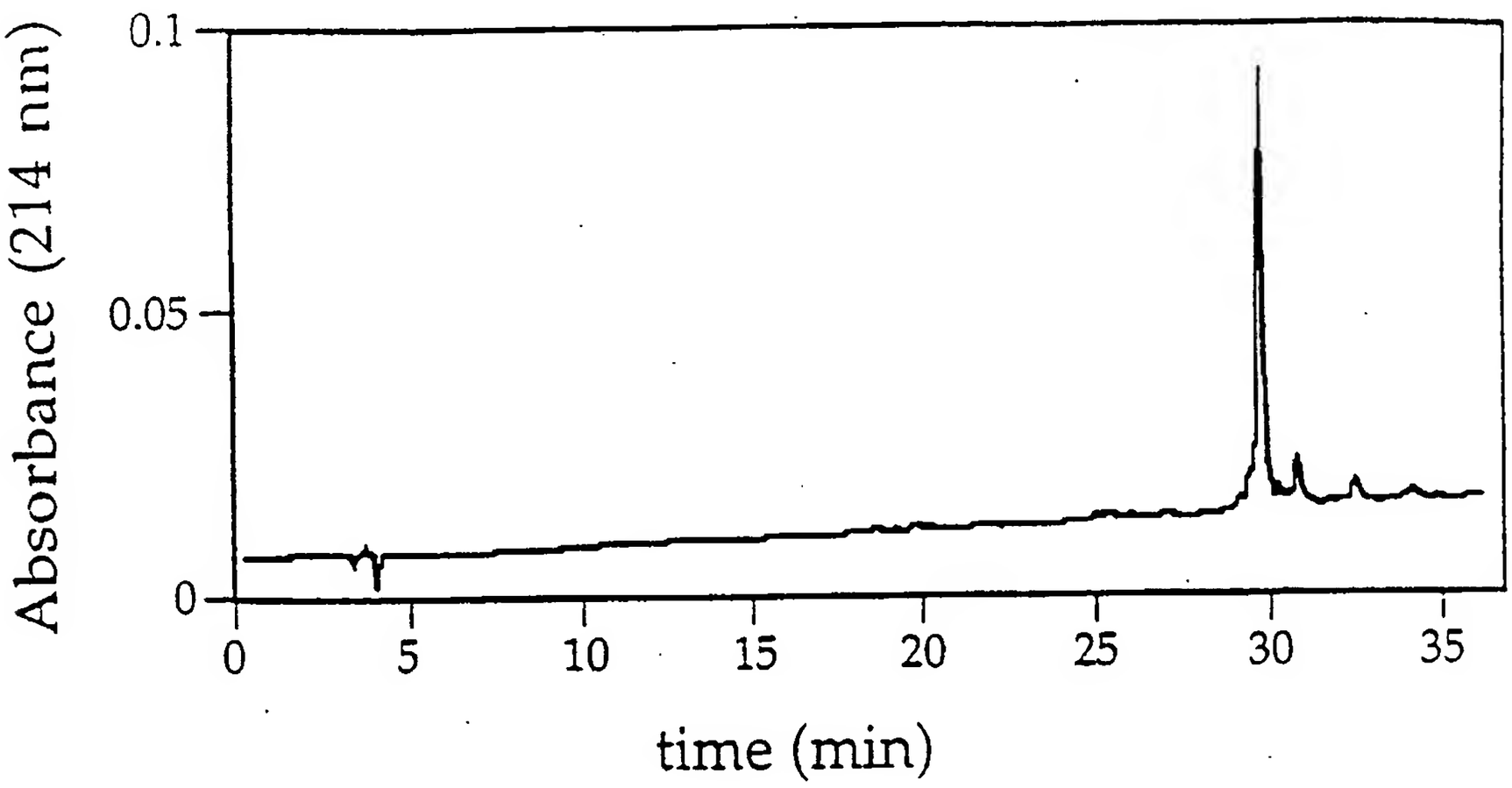


FIGURE 6

Competition ELISA of "binding" sequence vs. "binding/coding" sequence

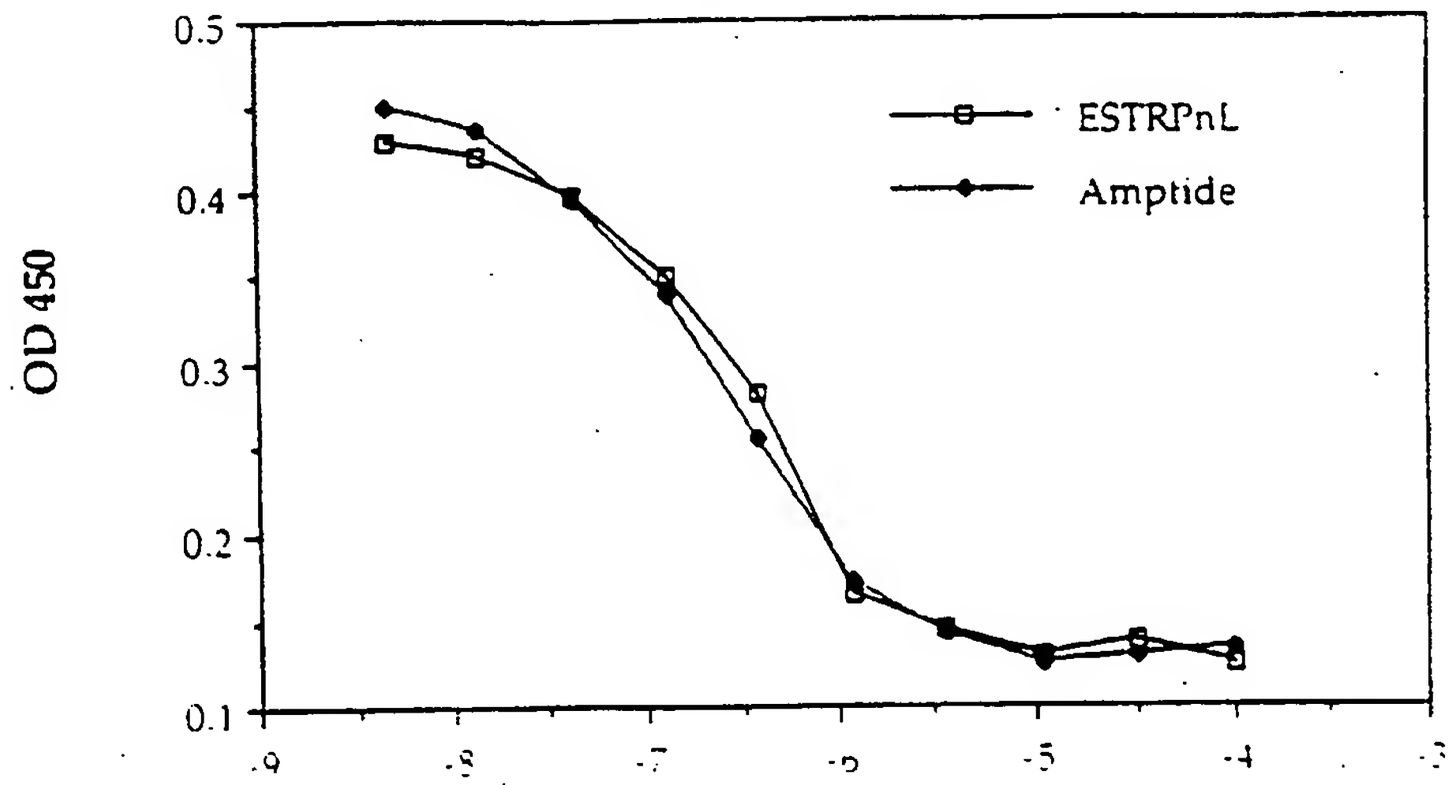
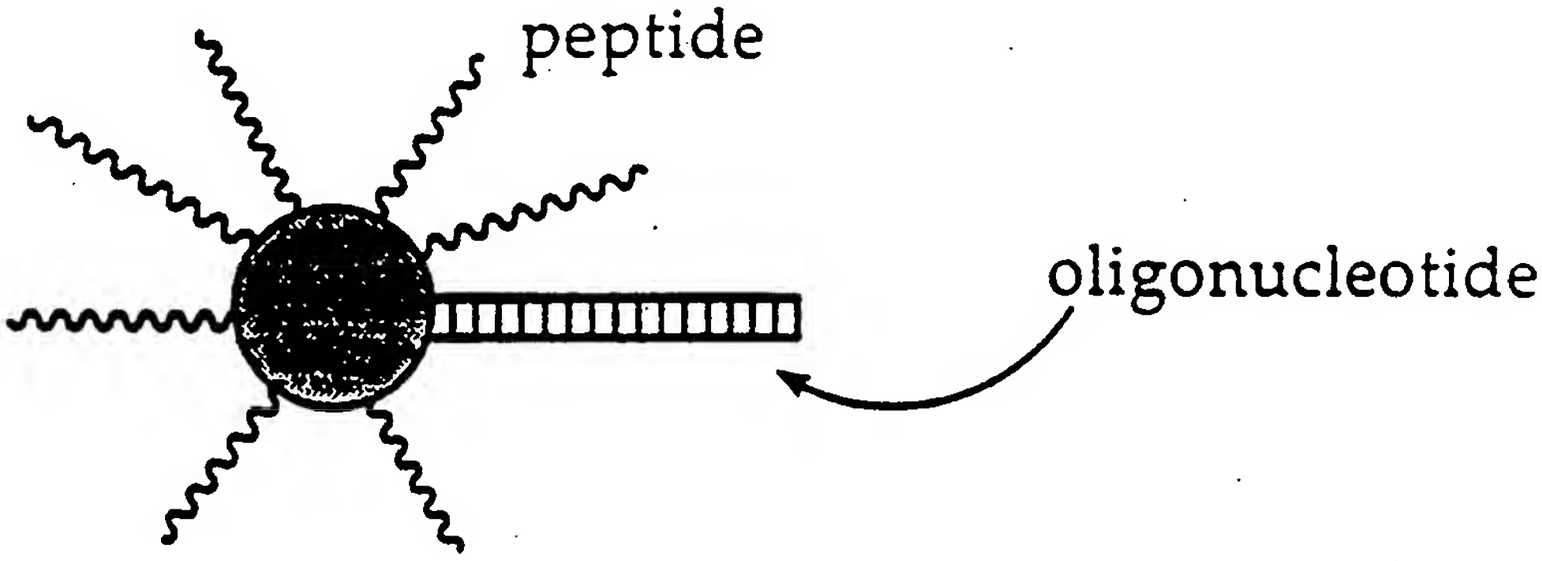


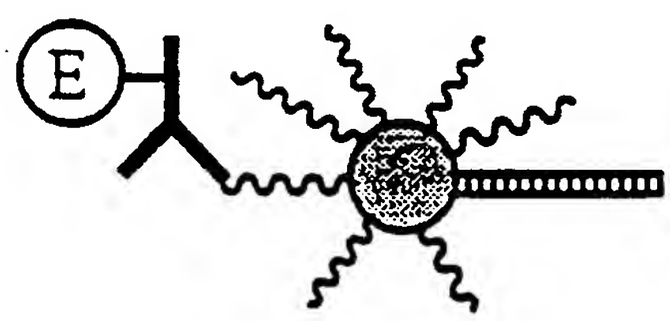
FIGURE 7

Analysis of solid-phase Amptides

- simultaneous synthesis of peptide & corresponding oligo



- detection/isolation of bead of interest



- DNA sequencing/amplification directly on a solid-phase*

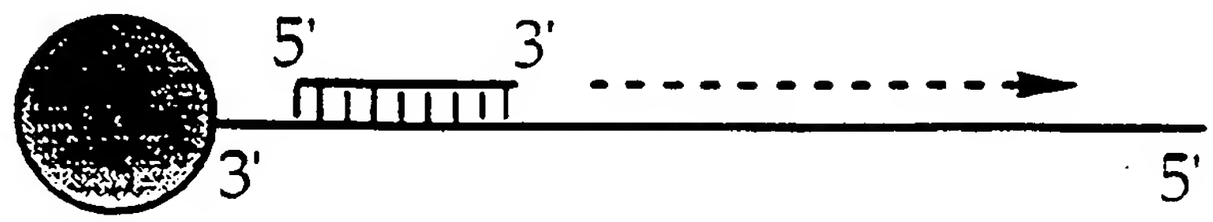


FIGURE 8

Analysis of solution-phase amptides

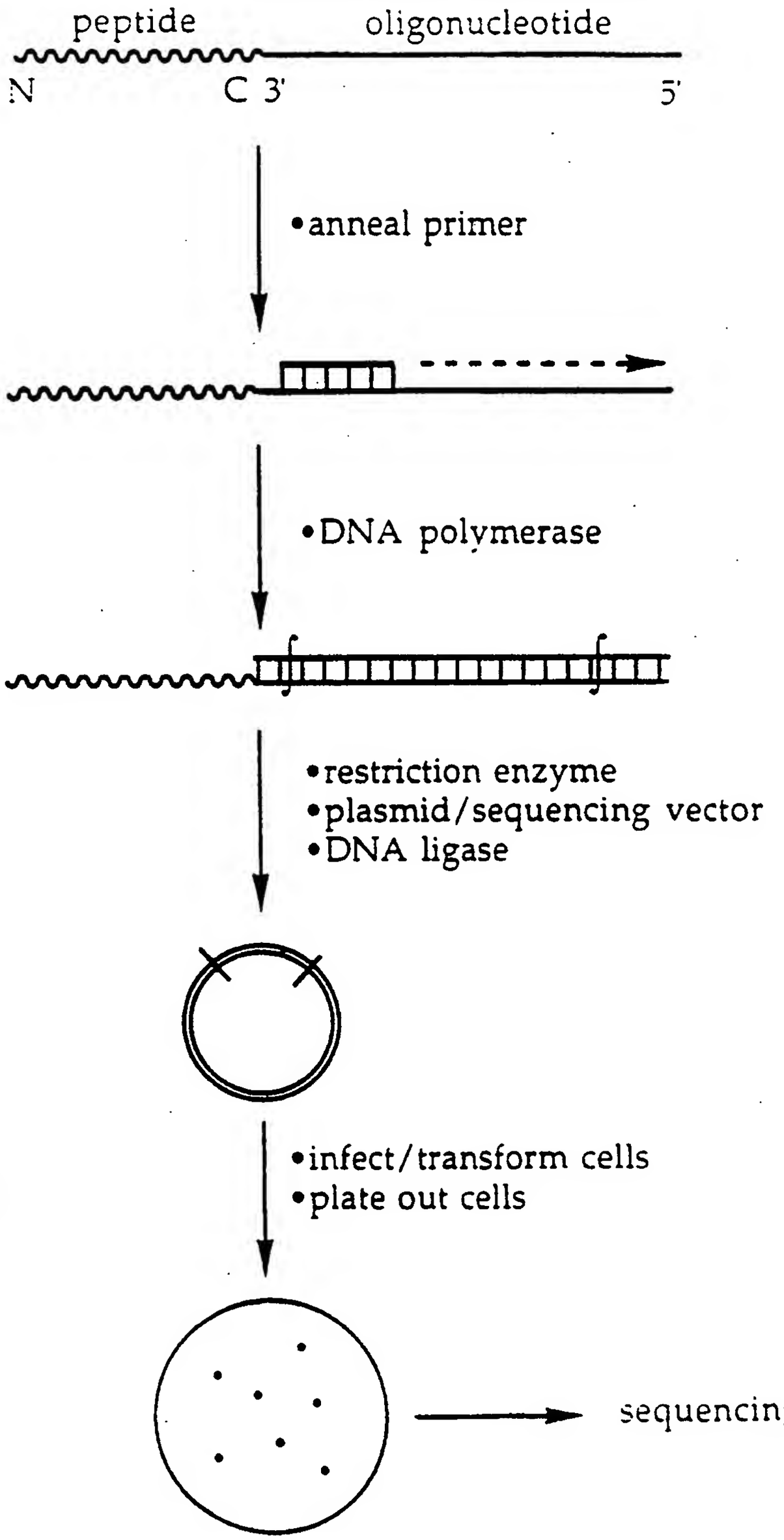


FIGURE 9

INTERNATIONAL SEARCH REPORT

Ir. ational application No.
PCT/US93/12013

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07C 237/00; C07H 21/00; C07K 1/00, 5/00, 7/00, 13/00, 17/00
US CL :530/300, 350, 333, 334, 338, 402; 536/25.3; 564/159

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/300, 350, 333, 334, 338, 402; 536/25.3; 564/159

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG
search terms: oligonucleotide, DNA, peptide, synthesis, combinatorial

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	Proceedings of the National Academy of Sciences U.S.A., Vol. 89, issued June 1992, S. Brenner et al., "Encoded combinatorial chemistry", pages 5381-5383, see the entire document.	1, 2, 4, 5, 8-12, 14, 15, 17-20 --- 3, 6, 7, 13, 16
X --- Y	Science, Vol. 257, issued 17 July 1992, I. Amato, "Speeding up a chemical game of chance", pages 330-331, see the entire document.	1, 2, 4, 5, 8-12, 14, 15, 17-20 --- 3, 6, 7, 13, 16

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 01 February 1994	Date of mailing of the international search report 24 FEB 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer ERIC GRIMES Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

In.. ..ational application No.
PCT/US93/12013

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P ---- Y, P	Proceedings of the National Academy of Sciences U.S.A., Vol. 90, issued November 1993, M. C. Needels et al., "Generation and screening of an oligonucleotide-encoded synthetic peptide library", pages 10700-10704, see the entire document.	1, 2, 4, 5, 8-12, 14, 15, 17-20 ---------- 3, 6, 7, 13, 16
Y	Science, Vol. 254, issued 06 December 1991, P. E. Nielsen et al., "Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide", pages 1497-1500, see page 1498.	6, 7
Y	BioTechniques, Vol. 13, No. 3, issued September 1992, R. A. Houghten et al., "The use of synthetic peptide combinatorial libraries for the identification of bioactive peptides", pages 412-421, see the entire document.	1-20
Y	US, A, 4,359,353 (KYDD) 16 November 1982, see columns 2-3.	1-20
A	Nature, Vol. 354, issued 07 November 1991, K. S. Lam et al., "A new type of synthetic peptide library for identifying ligand-binding activity", pages 82-84.	1-20
A	Science, Vol. 249, issued 27 July 1990, J. J. Devlin et al., "Random peptide libraries: a source of specific protein binding molecules", pages 404-406.	1-20

(12) UK Patent Application (19) GB (11) 2 295 152 (13) A

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C07K 1/04 , B32B 5/26 7/12 , D21H 17/26

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B5N N0526 N0712 N175 N176 N177 N178 N18X N207 N21X N22Y N224 N256 N26X N26Y N263 N2702 N2732 N401 N418 N420 N46X N476 N494 N566 N611 N648 N658 N66Y N661 N670 N672
D1R REX R151 R162 R318 R461 R513 R614 R658
D2W WA W326 W401 W412
U1S S1347 S1524 S1537 S1577 S1586 S1591

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Bioorg.Med.Chem.Lett. 1993,3(3),425-430 Peptide Research 1994,7(6),292-298 Drug Dev.Res. 1994, 33(2), 174-188

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UK CL (Edition N) **C3H HA3 HB4A HB4B**
INT CL[6] **C07H 21/00 , C07K 1/04**
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(54) Preparation of a library of compounds by solid-phase synthesis

(57) A method of making a library of compounds comprises the following steps:
(a) individually identifying a plurality of discrete reaction zones defined on laminar solid support material;
(b) charging each of said reaction zones with a starting material;
(c) sub-dividing the reaction zones into at least two initial batches;
(d) applying at least two different reagents, one to each of the reaction zones in each initial batch, and recording the identity of those reaction zones to which each of said different reagents is applied;
(e) subjecting all reaction zones to reaction conditions which promote reaction to completion;
(f) further sub-dividing the reaction zones into at least two alternative batches;
(g) applying at least two different reagents, one to each of the reaction zones in each alternative batch, and recording the identity of those reaction zones to which each of said different reagents is applied;
(h) subjecting all reaction zones to reaction conditions which promote reaction to completion, and
(i) repeating steps (f) to (h) inclusive as many times as desired.

The solid support may comprise paper prepared from cellulose comprising an amino group, obtained by reaction of the cellulose either with an amino precursor, followed by generation of free amine which is blocked by a protecting group, or with a compound having a protected amine group, followed by deprotection. The lamina may comprise a functionalised resin (aminomethylpolystyrene) sandwiched (with a polyethylene) between porous inert sheets (non-woven fibrous polypropylene).

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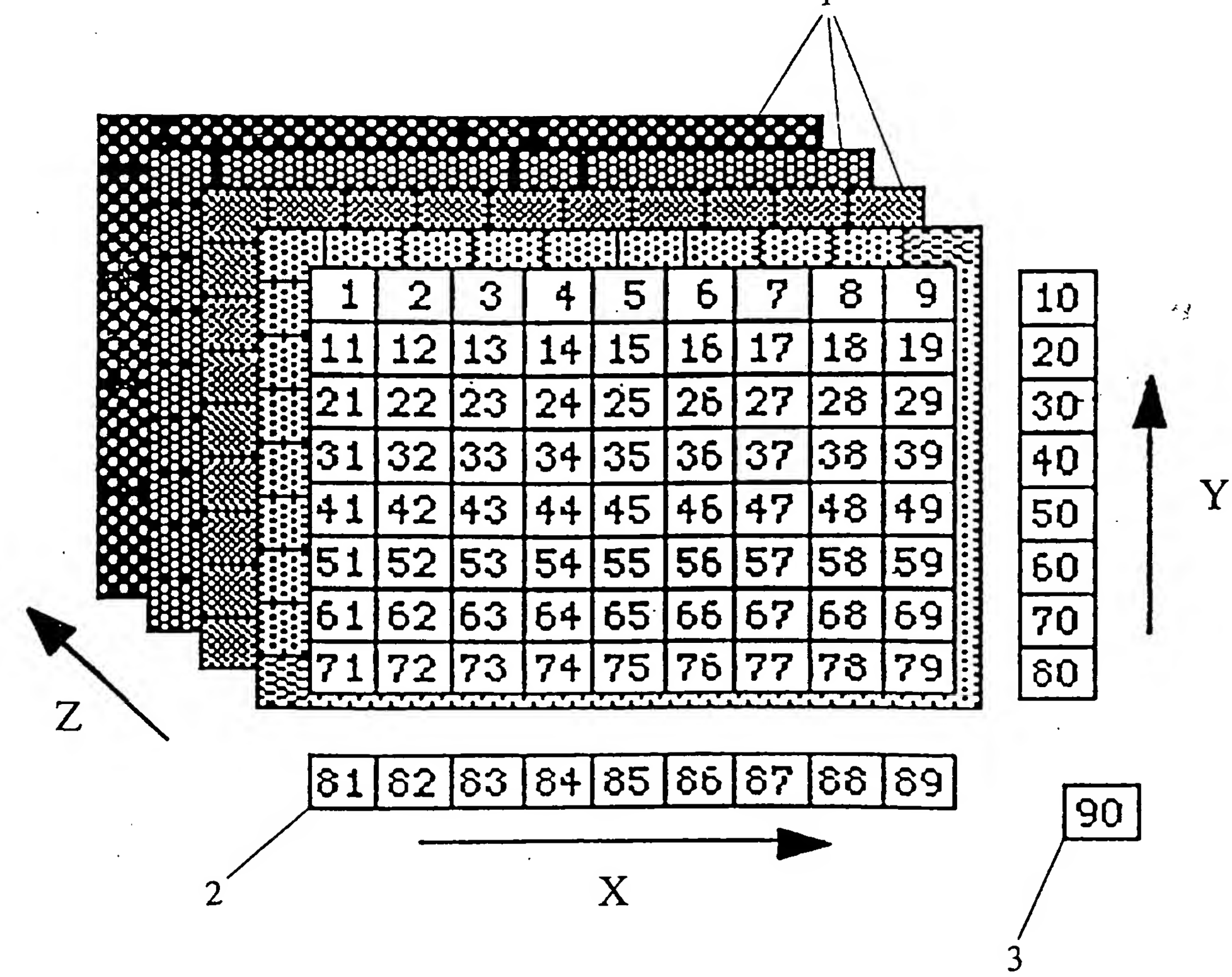
1
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61 62 63 64 65 66 67 68 69
71 72 73 74 75 76 77 78 79
10
20
30
40
50
60
70
80
Y
Z
81 82 83 84 85 86 87 88 89
90
2
X
3

FIGURE 1

METHOD OF MAKING A LIBRARY OF COMPOUNDS

The present invention relates to a method of preparation of chemical compounds and, in particular, to a method of preparing combinatorial libraries of chemical compounds. The method is especially suitable for the preparation of natural and synthetic chemical compounds which are to be tested for activity as therapeutic agents, though it need not be used exclusively for this purpose. In addition to being used for the preparation of combinatorial libraries, the method of the present invention also facilitates easy identification of individual compounds, so that any compounds which show encouraging biological activity can be prepared on a larger scale for further analysis. By modifying the method of the present invention, it is possible to prepare individual compounds in pure form in a non-combinatorial format.

The synthesis and screening of combinatorial libraries is becoming increasingly important in the pharmaceutical industry as a means of drug "discovery". The major advantages of combinatorial chemistry are that it is faster and cheaper than orthodox methods. This makes it a much more effective technique in the quest to uncover new therapeutic agents, particularly in circumstances where there is little or no information available concerning the types of structures likely to show the desired activity.

The wider availability of solid-phase synthetic methods has also led to increased interest in combinatorial chemistry. Clearly, solution chemistry is unsuitable for a technique which aims to produce a multiplicity of new products together, since this does not allow physical separation between the different materials produced. The products are therefore likely be contaminated with excess reagents, by-products etc, leading to difficulties in separation and purification.

The preparation of combinatorial compound libraries typically involves a number of successive stages, each of which involves a chemical or enzymatic modification of an existing molecule. Most typically, this process involves the addition of a monomeric unit or other synthon to a growing sequence, or the modification of chemical functionality on the sequence. Conveniently, the sequence or growing chain of interest is attached to a solid support. By carrying out the desired series of synthetic steps on the bound starting material, and by altering the nature of the monomeric or other synthon units, the type of chemistry and the sequence of reactions, it is possible to prepare an enormous number of individual compounds in short time.

As indicated above, combinatorial methods entail a series of chemical steps with multiple choices of chemical reagents for each step. The complexity of the combinatorial library thus produced is determined by the product of the number of reagent choices for each step of the synthesis, which can be quite large. The problem which then arises is identification and characterisation of members of the library which display particular desired properties.

Various solutions have been proposed to deal with this: For example, members of the library can be synthesised in spatially segregated arrays. However, because of the extra burden which maintenance of segregation imposes, this approach tends to lead to relatively small libraries. Alternatively, in the so-called "multivalent synthesis" method, a library of moderate complexity is produced by pooling multiple choices of reagents during synthesis. If a pool is shown to have properties of interest, it is re-synthesised with progressively lower complexity until a single compound or class of compounds is identified having the desired property. The ultimate size of a library produced by this technique is inevitably restricted because of concentration effects which determine the limits of detection at which activity can be discerned.

The so-called "mix and split synthesis" method relies on combinatorial synthesis carried out on discrete solid particles such as minute resin beads. Through a protocol of mixing and separating beads at the end of each step in the synthetic sequence, populations of beads are generated to which are bound the products of specific reaction sequences. Inevitably, individual beads obtained from the final reaction step have different products attached, so that identification and characterisation of active materials is still a problem.

Fortunately, biologically active compounds show remarkable potency and receptor sites are highly selective, so it is possible to detect low concentrations of active compound amid an extensive background of inactive material using standard *in vitro* screening techniques.

Another drawback of the mix and split synthesis method is that some measure of over-representation and omission of individual compounds is inevitable because of the randomness introduced by the mixing and splitting steps.

To counteract the above problems of identification and characterisation, some workers have proposed co-synthesis of a sequencable tag which encodes the series of steps and reagents used during synthesis of respective constituents of the library. More recently, it has been proposed to use tagging molecules to encode both the step number and the chemical reagent used in a given step, as a binary record of the synthetic steps experienced by each bead. This technique undoubtedly adds to the complexity of operations carried out during development of a combinatorial library.

From the foregoing, it is apparent that known methods of preparing combinatorial libraries of chemical compounds suffer from two major drawbacks: Either the materials are prepared by maintaining segregation, with the inevitable consequence that only relatively small libraries are practicable, or the materials are prepared without segregation but in such minute quantities that characterisation is rendered very difficult.

It is therefore an object of the present invention to provide a method of making a library of chemical compounds which allows wide diversification in the products obtained without over-representation and/or omission, at the same time as providing a clear indication of the sequence of steps which has been followed to synthesise a particular compound, thereby facilitating characterisation of individual materials.

In a first aspect, the invention is a method of making a library of compounds, which method comprises the following steps:

(a) individually marking with indicia a plurality of discrete reaction zones defined on laminar solid support material;

(b) charging each of said reaction zones with a starting material;

(c) sub-dividing the reaction zones into at least two initial batches;

(d) applying at least two different reagents, one to each of the reaction zones in each initial batch, and recording the identity of those reaction zones to which each of said different reagents is applied;

(e) subjecting all reaction zones to reaction conditions which promote reaction to completion;

(f) further sub-dividing the reaction zones into at least two alternative batches;

(g) applying at least two different reagents, one to each of the reaction zones in each alternative batch, and recording the identity of those reaction zones to which each of said different reagents is applied;

(h) subjecting all reaction zones to reaction conditions which promote reaction to completion, and

(i) repeating steps (f) to (h) inclusive from zero to n times, as desired.

It will be understood that n may be any whole number integer, the value of which depends on the complexity of the combinatorial library that it is intended to produce.

The method outlined above provides the synthetic chemist for the first time with the means to synthesise any number of single, easily identifiable labelled chemical compounds on a controllable pre-defined scale of preparation. In particular, this invention offers considerable handling advantages over prior art methods. For example, if desired the entire set of individual reaction zones may be handled as a single laminar medium. This opportunity does not exist with free-flowing microscopic resin beads. The method of division does not rely on the laminar support material being a particular shape. Thus, it is possible for the support to be in the form of tapes or streamers.

In an especially preferred form, the reaction zones are defined on sheets of material. An individual sheet may represent a single reaction zone, in which case a plurality of sheets is required to put the invention into effect. Alternatively, a single sheet may be sub-divided into an array of reaction zones of equal size, individual elements of the array being separable from each other for effecting step (c) above. In one possible variant of this method, each sheet is charged with a different starting material in step (b).

An especially preferred form of sheet material is paper, particularly paper which has been treated to enable the starting material to bind to the sheet. When the starting materials are amino acids or peptide fragments, the paper may for example carry allylic anchor groups to releasably bind the carboxylic acid groups of amino acids to the paper; a variety of other linking groups is also possible. The first and subsequent reagents may attach further amino acids or peptide fragments to the already bound amino acid residues on the sheet in known manner.

Another type of paper which may be used has free amino groups which may releasably bind to carboxy groups of amino

acids forming the starting material of the library compounds. One method of making such a paper is to treat cellulose, preferably in powder form, with acrylonitrile and a base, generally under aqueous conditions, to form a cyanoethyl ether of cellulose. The product may be dried and reduced, for example with borane in tetrahydrofuran, to aminopropyl cellulose. After removal of residual reagents the amino groups may be protected, for example by conversion of the aminopropyl groups to *tert*-butyloxycarbonyl aminopropyl groups and the resulting substituted cellulose may be mixed with cellulose fibre and formed into paper by standard paper-making methods.

The *tert*-butyloxycarbonyl or "Boc" groups may then be removed to provide the required paper with free amino groups.

In a second aspect, the invention is a method of preparing a paper support material for use in the synthesis of chemical compound libraries, which method comprises:

(a) linking cellulose with a compound which is selected from the set consisting of an amine precursor or a compound having a protected amine group;

(b) in the case of an amine precursor, generating the free amine and then protecting it with a conventional amino protecting group;

(c) incorporating the amine-functionalised cellulose into a paper sheet by mixing with paper fibre and forming into sheets, and

(d) reacting the paper sheets obtained from step (c) above with an amino deprotecting reagent to provide free amine groups on the paper sheets.

Alternatively, materials other than paper may be used for making the sheets. This is an important consideration for those branches of chemistry which require a non-protic environment, since paper is a protic material.

One possible alternative is a polyethylene or polypropylene film which has been grafted with polystyrene chains, as described in published PCT Patent Application No. WO 90/02749. Alternatively, the sheet may be of a laminated construction, being in the form of a solid material trapped between two or more layers of porous mesh. One laminate of this type consists of a so-called "resin cloth" comprising cross-linked polystyrene resin containing amino groups formed as a layer sandwiched between fibrous sheets, for example, non-woven polypropylene sheets, on which indicia may be borne. The use of other materials is, of course, possible.

A non-protic sandwich material such as that described above permits a wider range of chemistries to be carried out. For example, chemistry is permitted to be performed on a supported resin cloth which usually requires strictly anhydrous conditions. Example reactions include, but are not limited to, use of a strong non-protic base to generate anions of chemical substrates affixed to the resin cloth. Further manipulations of these anions permits, for example, Heck type couplings, Stille couplings, heteroaryl couplings, carbonylations, carboxylations and carbamoylations not normally permitted in a protic environment.

In a third aspect, the invention is a method of preparing a laminar resin support material for use in the synthesis of chemical compound libraries, which method comprises affixing a layer of particulate functionalised solid support resin material to a porous inert laminar material.

Preferably, the layer of particulate functionalised solid support resin material is sandwiched between two layers of porous inert laminar material.

In general, suitable sheet material may be any material which is readily markable with indelible indicia, is divisible in equal proportions, allows the sheets to be formed into a stack and subsequently separated and to which the constituents of the compounds of the library may releasably be bound. The sheet and method of binding the compounds are preferably such

that known amounts of the compound may be repeatably released from a single sheet portion bearing the compound.

It should be noted that the present invention is not limited to the preparation of biologically active compounds. It is applicable to any organic or inorganic species which, used in combination with other reagents, will form oligomers bound to the solid sheet. The compounds which are stored in the library must however be compatible with the material of the sheet.

Besides sheet materials, any suitable support material can be adapted for use in the method of the present invention provided that it has the capacity for division and subsequent sub-division into discrete reaction zones and provided that it possesses the necessary surface active qualities to serve as a vehicle for the intended reaction steps.

The compounds prepared in the library may be linked to the support by a wide variety of methods, depending on the nature of the support and the compounds to be prepared. Apart from the allylic anchoring group/amino acid system mentioned above, chemical linkers which may be cleaved by acidic, basic, hydrogenolytic or other chemical reagents may be used, as may light-induced cleavage. Combinations of these methods may also be used.

The amount of compound stored in each reaction zone may vary according to the nature of the compound and the nature of the support material, and also according to the size of the zones. Amounts of compound varying from a few nanograms to several milligrams may be stored on portions of paper sheet of convenient size. In principle any amount of compound may be stored provided that the support material is large enough.

Typically, the different reagents used in the method according to the present invention are individual monomeric units and may be chosen from a large variety of compounds. These include agents such as amino acids, nucleotides, sugars, naturally-occurring and synthetic heterocycles, lipids, and

combinations thereof, though it will be understood that this list is not exhaustive. In general, any bifunctional group may be used which may be linked to the support material or to the growing sequence in protected form, and subsequently deprotected and reacted with a further group. Alternatively, a monofunctional group may be used to complete the sequence.

It is an essential feature of the present invention that individual reaction zones are identified, that is to say, labelled with some form of indicia which uniquely characterises each reaction zone. The indicia may comprise, for example, numbers, letters, symbols or colours in a coded combination. The indicia may be applied to the respective reaction zones before synthesis commences using known printing methods. These are preferably such that the ink used will not leach out of the reaction zones during the synthetic procedures, or otherwise interfere with formation and subsequent removal of a compound held on a particular reaction zone. U.V.-sensitive ink which is "fixed" to the reaction zones by exposure to ultraviolet radiation after printing is generally suitable for this purpose. Other types of indicia, not necessarily optical in nature, may be used for identifying individual reaction zones. Possible alternatives include Smiles strings, bar-codes, chemical structures, marked or printed punched card formats, ultraviolet-readable fluorescent systems and electro-magnetically readable devices such as magnetic strips. The type of indicia used may depend on the size and shape of the support material and/or reaction zones.

The invention will now be described by way of example only with reference to the drawing (Figure 1) which shows in schematic form one particular embodiment of support material used in performance of the present invention and a convenient pattern of sub-division.

Referring now to Figure 1, the illustrated arrangement shows orthogonal arrays of reaction zones 3 defined on a series of support sheets 1. The reaction zones are arranged in a grid or matrix layout in straight rows along one

dimension and straight columns along the other dimension, each reaction zone being provided with a unique tag or label.

In the next step, each of the sheets 1 is treated with a different first reagent which becomes bound to the sheet to form the first monomer or constituent serving as the starting material for subsequent steps. The sheets are then superposed to form a block in which corresponding reaction zones 3 of respective sheets are aligned with each other. The block of sheets so formed is then divided by making a first series of cuts through the stack, e.g. in the X direction, thereby forming a plurality of stacked strips 2.

Each stack of strips 2 is then treated with a reagent to effect deprotection or activation of the first constituent following reaction with a different second reagent to effect binding of a respective second constituent to the first constituents already bound on the strips.

Following this, the treated stacks of strips are reassembled to reform the block and a second series of cuts is made at right angles (in the Y direction) to the first so that each strip becomes further sub-divided into smaller elements corresponding to the reaction zones (3).

Each of the stacks of individual reaction zones is then deprotected if necessary and treated with a different third reagent to effect binding of a respective third constituent on the free end of the second constituent already in place.

If, in this example, a total of twenty sheets is used initially and if each sheet is treated with a different first reagent monomeric unit, twenty different sheets having attached a first monomer or fragment will be formed. When the superposed sheets are divided to form, say, 20 strips and each strip is treated with a different reagent a total of 20x20=400 dimeric chains is formed, each having a different combination of first and second monomers or fragments. Subsequent reassembly of the block and further sub-division along the second dimension into, say, 20 slices and treatment of each of the slices with different reagents will give 20x20x20=8,000

different pure trimeric structures. The total number of monomers, dimers and trimers may be increased by dividing the block into a greater number of strips or slices, or by increasing the number of sheets. Thus, if 50 sheets are used, divided into 50 strips and 50 slices, the number of different pure individual trimeric structures will be 125,000.

Each of the trimers will be different and will be identifiable unambiguously from the indicia (which may be letters and/or numbers applied by printing) marked on the reaction zones.

At this final stage of the process, the sheets have been cut in a fashion to provide individual pieces of paper, each of which is marked with a single, unique index, which is in itself an identifier of the single, unique chemical structure attached to that portion of paper. Furthermore, all possible combinations are formed of compounds available from the constituents provided by the reagents used.

In the embodiment of the invention described above, the reaction zones may be square or oblong and arranged in an orthogonal pattern. However, other geometrical arrangements may be used. In principle the sheet portions may be of any shape and arranged in any type of grid pattern, subject only to the need to divide the sheet into individual portions.

The sheet material may be, but is not limited to, paper and depending on the size of the sheets cutting may be carried out using any suitable cutting device, such as scissors or an ordinary office guillotine. The arrangement described above allows a very large number of different dimeric and trimeric and larger polymeric structures to be assembled easily and rapidly.

It will be apparent that in a library of single individual compounds, each of which is identified by means of its own unique indicia, an individual sheet portion may be easily identified. Thus, evaluation of the biological or other activity of the compound cleaved from such an identified sheet portion will permit, by means of targeted screening of

The method of making a library of compounds described above may be considered as starting with a three-dimensional stack of sheets which is divided three times in different dimensions (once by separating the sheets, and twice by cutting) and treating with three different sets of reagents. The same principle may be applied to a two-dimensional system using a single sheet which is divided twice in two transverse directions and treated with two reagents.

Such an arrangement still allows provision of a large number of compounds in a library. For example, if a single sheet is divided into a pattern consisting of 50x50 squares a total of 2500 different compounds, each of known composition and unambiguously identified, may be obtained. In another embodiment, the sheets may be in the form of tape or streamers which comprise only a single line of reaction zones which are separated by cutting in the transverse direction. These tapes or streamers bearing a one-dimensional array of reaction zones may be superposed to form a block which is treated and subdivided in a manner similar to that described above.

It will also be appreciated that the block of sets of individual reaction zones may be further divided and reacted with a fourth or subsequent set of reagents to provide a further dimension of product variation.

In general, the invention is applicable to any arrangement of sheet material in which both the "sheets" and the reaction zones defined thereon may, after sub-division, be handled and subjected to the desired chemical process steps without losing their physical integrity or their identifying indicia. The manner in which the sheets are divided into portions (cutting, stamping, tearing etc.) will depend on the identity of the sheet material and the shape and size of the reaction zones.

The invention is further illustrated by the non-limiting examples described below, in which the following abbreviations are used:

Fmoc: 9-Fluorenylmethoxycarbonyl

Boc: Tert-butyloxycarbonyl

THF: tetrahydrofuran

DMF: N,N-dimethylformamide

TFA: trifluoroacetic acid

HOBt: N-1-hydroxybenztriazole

TBTU: 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate

Hunig's base: N,N-diisopropylethylamine

EXAMPLE 1

Syntheses conducted on Boc-aminopropyl cellulose sheet

Preparation of cyanoethyl cellulose:

A suspension of 70% water wet partially cross-linked cellulose powder (XEC Whatman) 13kg was suspended in dioxan (28l), and treated with a solution of sodium hydroxide (210g) in water (200ml) and the viscous suspension stirred at room temperature for 10 min. Acrylonitrile (201.5g, 250ml, 3.79 mole) was added, the mixture stirred 5 min, further acrylonitrile (201.5g, 250ml, 3.79 mole) was added, the mixture stirred 5 min, and thereupon a final portion of acrylonitrile (403g, 500ml, 7.95 mole) was added and the whole reaction mixture stirred at room temperature for a total of 5hr. There was no detectable exotherm under these conditions.

The bulk material was recovered by filtration, and the crude product washed with water until the washings were of pH 7. The water was then removed by suction, the filter cake dried by suspension in acetone (2 x 10l), collected by filtration, further washed with acetone (2 x 10l), and finally

dried at 80°C for a total of 72hr. A total of 3.78kg anhydrous material was obtained. Elemental analysis of the recovered solid shows there to be N present in the expected ratio.

CHN analysis: Found % C: 46.60; H, 6.60; N, 2.16
C: 46.52; H, 6.54; N, 2.13

This experiment was repeated three times on approximately the same scale to provide a total of 11.9kg of dried sample of the cyanoethyl ether of cellulose.

Reduction of cyanoethyl cellulose to aminopropyl cellulose:

A dry sample of cyanoethyl cellulose powder as above was purged under dry nitrogen, treated cautiously with a solution of borane/tetrahydrofuran complex (1M) in THF (14l), stirred for 1hr at room temperature, and then cautiously warmed to gentle reflux for a total of 24hr. The cooled solution was very cautiously treated aqueous ethanol (10%, 1l) with external ice-water cooling, and some evolution of hydrogen was detected. The wet slurry was then filtered and the wet filter cake slurried in HCl (1M, 12l) for a total of 30min, recovered by filtration, and re-suspended in (1M, 12l) for a total of 1hr. The product was collected by filtration, washed extensively with water until the washings were of pH 7, and then sucked dry. This filter cake was then slurried in ethanol (10l), collected by filtration and sucked dry for a total of 1hr. This cake was then slurried with ether (10l), the product collected by filtration, sucked dry overnight at room temperature and was finally dried at 50°C to constant weight.

Analysis of the free amine content by standard methods revealed an amine content of 0.50 mmole/g dry weight.

This experiment was also repeated for a total of three times to provide a total of 13.24kg of dry powder.

Protection of the amino group to give Boc-aminopropyl cellulose:

A solution of sodium carbonate (1.26kg) in water (1l) was diluted with THF (12.1) and a sample of aminopropyl cellulose hydrochloride (4kg) was added cautiously to avoid frothing. This was treated with di-*tert* butyl pyrocarbonate (4kg) and the mixture was allowed to stand at room temperature for one week. The solid material was collected by vacuum filtration, washed with water until the washings were of pH 7, then slurried in acetone (10l), and collected by filtration. This slurry treatment of the collected solid was then repeated (10l). The product was finally collected by filtration, sucked to dryness and dried overnight at 80°C under vacuum. This gave a colourless solid 4.2kg.

This experiment was then repeated three times to provide a total weight of the N-protected derivative of 13.06kg. This showed a residual moisture content of approximately 15%, which could have been removed by extremely vigorous drying. However, such removal was unnecessary for the next step in the procedure.

Preparation of bulk scale Boc-aminopropyl cellulose paper sheet:

Blank paper fibre in the form of long staple raw cellulose (27.8kg) was slurried in a large volume of water (2600 litres) for a total of 20 min. This slurry was combined with the sample of powdered Boc-aminopropyl cellulose (13.06kg) and further slurried for a total of 10 min to achieve adequate dispersion. A polyamide epichlorohydrin cross-linking agent (1.14 l) was added, and the paper slurry was then prepared in sheet form by conventional means. This produced a finished roll of paper of approximately 28kg in weight.

Deprotection of the Boc-aminopropyl cellulose sheet:

A sample of the Boc-aminopropyl cellulose sheet of A4 size was suspended in a solution of trifluoroacetic acid in solution of dichloromethane (50%, 30ml), for a total of 30 min. The paper was then washed with dimethylformamide (DMF) to remove excess TFA, with methanol (x 1), neutralised (1M NaOH), washed water, methanol and then dichloromethane and finally dried at 40°C under vacuum for a total of 1hr.

This paper was assayed for free amine content by a known method using picric acid, which showed a reproducible free amine level in the range of 2-3 nmoles/mm^2.

Preparation of Lys-Tyr-Lys and Thr-Tyr-Ser on amine-functionalised paper:

Fmoc-O-t-butyl-Ser Derivatisation:

A sample of the above described amine functionalised paper (two sheets of dimensions 210 x 197 mm, of 2.85 nmoles/mm^2, or 0.18 mmole total amine content) indelibly marked with indicia was derivatised by reaction with 2,4-dichlorophenyl-4-(N-α-Fmoc-O-tert-butyl-Serinyloxymethyl) phenoxyacetate (650mg or 2.7 times excess) in DMF solution for a period of 17 hr at room temperature following the general method given by Bernatowicz *et al*. (Tetrahedron Letters 1989, 30: 4341). The paper was washed with DMF to remove reagents (x 3), dichloromethane (x 6) and was then dried at room temperature under vacuum.

Determination of free amine content indicated that the degree of coupling in this reaction was of the order of 85%.

Residual amine groups were acetylated using a solution of acetic anhydride (4ml), collidine (6ml) and 4-dimethyl-aminopyridine (2g) in acetonitrile (20ml) for 1hr at room temperature. The paper was then washed with acetonitrile (x 3), dichloromethane (x 6), and dried under vacuum.

Deprotection of the Fmoc group was achieved using a standard method utilising a solution of piperidine (15ml) in dichloromethane (5ml) for 30min at room temperature. Washing of the paper with DMF (x 2) and dichloromethane (x 6), followed by drying under vacuum gave the material ready for the next coupling.

α-Fmoc-ω-Boc-Lys Derivatisation:

A sample of the same amine derivatised paper of identical dimensions indelibly identified with indicia was derivatised in an identical fashion using instead the analogous derivative of α-Fmoc-ω-Boc-Lys.

Residual amine acetylation and deprotection prior to the further functionalisation were performed in a manner identical to that described above.

Coupling of the second monomer (Fmoc-O-*t*-butyl-Tyr):

The two above monomerically linked pieces of paper were placed in one vessel and treated with a five-fold excess of a solution of the HOBt ester of Fmoc-Tyr-O-*t*-butyl ether which was prepared by pre-activation of a solution of Fmoc-O-*t*-butyl-Tyr (3.47g, 7.56 mmol), HOBt (1.02g, 7.56 mmol), TBTU (2.42g, 7.56 mmol) and Hunig's Base (2.64ml, 15.12 mmol) in DMF (160ml) for a period of 30min. This preformed ester solution was then reacted at room temperature overnight with the paper samples. The pieces of paper were then washed with DMF (x 3) to remove reagents, dichloromethane (x 6), and dried under vacuum.

Residual amine groups were acetylated using a solution of acetic anhydride (4ml), collidine (6ml) and 4-dimethyl-aminopyridine (2g) in acetonitrile (20ml) for 1hr at room temperature. The paper was then washed with acetonitrile (x 3), dichloromethane (x 6), and dried under vacuum.

Deprotection of the Fmoc group was achieved using a standard method utilising a solution of piperidine (15ml) in DMF (15ml) per sheet for 30min at room temperature. Washing

of the paper with DMF (x 4) and dichloromethane (x 6), followed by drying of the paper under vacuum gave the material ready for the next coupling.

Coupling of the third monomer:

The sample of paper bearing Tyr-Ser was further reacted separately with a sample of the HOBt ester of Fmoc-Thr-O-*t*-butyl ether prepared by pre-activation of a solution of Fmoc-Thr-O-*t*-butyl ether (1.5g, 3.78 mmol), HOBt (0.51g, 3.78 mmol), TBTU (1.21g, 3.78 mmol) and Hunig's Base (1.31 ml, 7.56 mmol) in DMF (85ml) for a period of 30 min. The paper was reacted at room temperature overnight.

The sample of paper bearing Tyr-Lys was further reacted separately with a sample of the HOBt ester of α-Fmoc-Boc-Lys prepared by pre-activation of a solution of α-Fmoc-Boc-Lys (1.77g, 3.78 mmol), HOBt (0.51g, 3.78 mmol), TBTU (1.21g, 3.78 mmol) and Hunig's Base (1.31ml, 7.56 mmol) in DMF (85ml) for a period of 30 min. The paper was reacted at room temperature overnight with this preformed solution.

The two pieces of paper were then washed with DMF (x 2) to remove reagent, dichloromethane (x 3), and dried under vacuum. Residual amine groups were acetylated using a solution of acetic anhydride (4ml), collidine (6ml) and 4-dimethylaminopyridine (2g) in acetonitrile (20ml) per sheet for 1hr at room temperature. The paper was then washed with acetonitrile (x 4), dichloromethane (x 4), and dried under vacuum.

Final deprotection of the Fmoc group was achieved using a standard method utilising a solution of piperidine (15ml) in DMF (15ml) per sheet for 30min at room temperature. Washing of the paper with DMF (x 4) and dichloromethane (x 6) followed by drying of the paper under vacuum gave the material ready for the final cleavage.

Cleavage of the trimers from the paper:

The sample of paper bearing Thr-Tyr-Ser was cut into small portions, treated with TFA/H_2O (95:5, 89.25ml) and stored at room temperature overnight. Solid material was removed by filtration and washed with dichloromethane (x 2) and methanol (x 2), and the filtrates combined. Acid was removed by evaporation below 40°C, and the sample freed from acid by azeotropy from toluene/dichloromethane (x 2). The sample was dissolved in water (15ml), filtered, and freeze dried. The mass recovery was essentially quantitative. This was examined by hplc analysis and the desired material was shown to be the major product by comparison with a genuine sample, and moreover exhibited identical m/e peaks in the mass spectrum.

The sample of paper bearing Lys-Tyr-Lys was cut into small portions, treated with TFA/H_2O (95:5, 89.25ml) and stored at room temperature overnight. Solid material was removed by filtration, washed with dichloromethane (x 2) and methanol (x 2), and the filtrates combined. Acid was removed by evaporation below 40°C, and the sample freed from acid by azeotropy from toluene/dichloromethane (2 x). The sample was dissolved in water (15ml), filtered and freeze dried. The mass recovery was essentially quantitative. This was examined by hplc analysis and the desired material was shown to be the major product by comparison with a genuine sample, and moreover exhibited the desired m/e peak in the mass spectrum.

Preparation of a 1677 component peptoid library for biological screening:

Three sheets of amine functionalised paper as described above of dimensions 210 x 297mm were indelibly marked with a pattern of indicia (43 columns and 39 rows), and subsequently deprotected with TFA in the manner described above ready for the coupling of the first monomers. Analysis revealed the presence of 1.9 nmol/mm^2 of amine groups.

Functionalisation of the sheet:

Each of the 43 columns of paper was divided from the original sheet and separately functionalised as follows: Each column of paper was treated with an individual Fmoc-protected amino acid derivative pre-activated as its 2,4-dichlorophenyl 4-(oxymethyl) phenoxy acetate as described above in a solution of DMF (0.5ml) and pyridine (10ml) at room temperature overnight. The paper was washed with DMF (x 4) and dichloromethane (x 5) and dried at 40°C for 30 min. Acetylation of residual amine functionality was carried out as described above.

Deprotection of the amine groups was also carried out as described above.

Coupling of the second monomer:

The complete set of stacked strips of reaction zones of the original paper sheets was assembled into one block and then cut again at right angles to the original cutting direction into individual reaction zones. Each set of individual reaction zones from a complete row was then coupled with a second Fmoc-protected monomeric unit, pre-activated as its 2,4-dichlorophenyl 4-(oxymethyl)phenoxy acetate ester as described above. On completion of reaction, these individual reaction zones were washed, acetylated, and finally the Fmoc protection group was removed as described above.

Coupling of the third monomer:

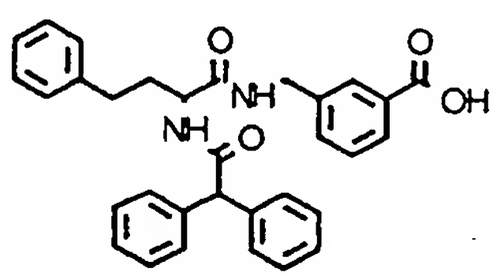
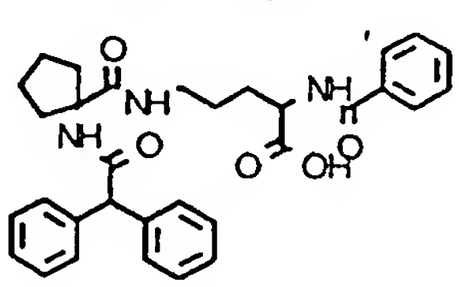
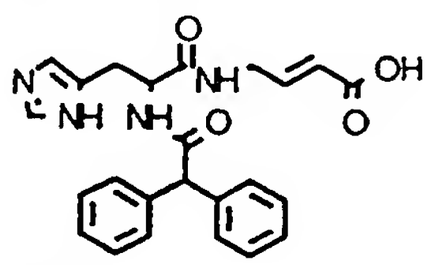
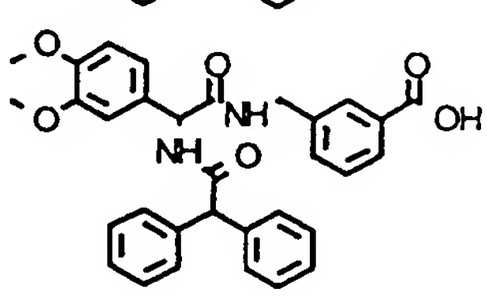
The complete set of 1677 individual reaction zones charged with dimeric amine derivatives were combined into one vessel, and reacted with diphenylacetyl chloride (2.31g, 0.1 mol), with Hunig's Base (3.5ml, 0.1 mol) in DMF (96.5ml) at room temperature overnight. The set of individual reaction zone was washed with DMF (x 3), and dichloromethane (x 4) and dried at 40°C for 30 min under vacuum. To remove all extraneous reagents, the complete set of reaction zones was treated in a Soxhlet extractor with dichloromethane overnight,

and the extract discarded. The reaction zones were dried at 40°C under vacuum for three hours.

Cleavage of individual trimeric products:

The trimeric products were removed from the paper in the following manner: Each individual labelled reaction zone was separated and treated with TFA/H_2O (95:5, 50ml) at room temperature overnight. Each reaction zone was then washed with dichloromethane (12 x 50ml), methanol (4 x 50ml), the washings being combined and evaporated under nitrogen. Analysis of individual products is exemplified by the following: After cleavage from the paper support, individual trimeric products were identified by the indicia marked thereon. A subset of these was examined by both hplc and mass spectrometry and, in the cases examined, confirmed the presence of the desired compound.

Below is a subset of typical analytical data for compounds examined by mass spectrometry:

Indicia	Structure	Expected m/e	Found m/e
A3410		533.62	535.0
B3315		541.62	543.0
C3702		432.41	432.8
A0604		538.61	539.0

Example 2

Syntheses on aminomethyl-substituted laminated resin sheet

Preparation of aminomethyl-substituted laminar sheet:

A sample of 100g of a partially cross-linked aminomethyl polystyrene resin (Novabiochem 01-640010) was thoroughly mixed with a sample of low melting thermoplastic polyethylene glue (Dritex DT157/300) and the mixture was evenly spread over the surface of a portion of non-woven fibrous polypropylene sheet (Freudenberg Lutrasil 4150) of area 16 square metres. A further sheet of the same non-woven fibrous polypropylene sheet of identical area was then superimposed onto the bottom loaded sheet, and the two were then heat welded together (within a temperature range of 90-140°C) to give a single material containing resin in which amino groups were demonstrably available.

Titrimetric analysis of the free amine content showed that, in this example, free amine density was 2.2 nmole/mm^2.

Fmoc-O-*t*-butyl-Ser Derivatisation:

A sample of the above polypropylene cloth (210 x 145 mm, or 67.8 mmole total amine content), indelibly identified with indicia, was derivatised by reaction with the 2,4-dichlorophenyl N-α-Fmoc-O-t-butyl-Ser-4-oxymethylphenoxyacetate ester (258mg or 5 times excess) in DMF solution for a period of 17hr at room temperature. The resin cloth was washed with DMF (x-3) to remove reagents and dichloromethane (x 3), and was then dried at room temperature under vacuum.

Residual amine groups were acetylated using a solution of acetic anhydride (4ml), collidine (6ml) and 4-dimethylaminopyridine (2g) in acetonitrile (20ml) for 1hr at room temperature. The resin cloth was then washed with acetonitrile (x 3), dichloromethane (x 6), and dried under vacuum.

Deprotection of the Fmoc group was achieved using a standard method utilising a solution of piperidine (10ml) in DMF (10ml) for 30min at room temperature. Washing of the cloth with DMF (x 4) and dichloromethane (x 6), followed by drying under vacuum gave the material ready for the next coupling.

Fmoc-α-Boc-Lys Derivatisation:

A sample of the same polypropylene cloth of identical dimensions, indelibly identified with indicia, was derivatised in an identical fashion using instead the analogous derivative of Fmoc-α-Boc-Lys.

Residual amine acetylation, and deprotection prior to further functionalisation were performed in a manner identical to that described above.

Coupling of the second monomer:

The two above monomerically linked pieces of resin cloth were combined in one vessel and treated with a five-fold excess of a solution of the HOBt ester of Fmoc-O-t-butyl Tyr prepared by pre-activation of a solution of Fmoc-O-t-butyl Tyr (331mg, 0.68 mmol), HOBt (92mg, 0.68 mmol), TBTU (217mg, 0.68 mmol) and Hunig's Base (120ml, 0.68 mmol) in DMF for a period of 30min. This pre-activated ester was then reacted with the pieces of resin cloth at room temperature overnight. The two pieces of resin cloth were then washed with DMF (x 3) to remove reagents, dichloromethane (x 6), and dried under vacuum.

Residual amine groups were acetylated using a solution of acetic anhydride (4ml), collidine (6ml), and 4-dimethyl-aminopyridine (2g) in acetonitrile (20ml) for 1hr at room temperature. The resin cloth was then washed with acetonitrile (x 3) and dichloromethane (x 6), and dried under vacuum.

Deprotection of the Fmoc group was achieved using a standard method utilising a solution of piperidine (10ml) in

DMF (10ml) for 30min at room temperature. Washing of the cloth with DMF (x 4) and dichloromethane (x 6), followed by drying of the resin cloth under vacuum, gave the material ready for the next coupling.

Coupling of the third monomer:

The sample of resin cloth bearing Tyr-Ser was further reacted separately with a sample of the HOBt ester of Fmoc-O-t-butyl-Thr prepared by pre-activation of a solution of Fmoc-O-t-butyl Thr (134mg, 0.339 mmol), HOBt (46mg, 0.339 mmol), TBTU (108mg, 0.339 mmol) and Hunig's Base (60ml, 0.339 mmol) in DMF (10ml) for a period of 30 min. The resin cloth was reacted at room temperature overnight with this preformed reagent.

The sample of resin cloth bearing Tyr-Lys was further reacted separately with a sample of the HOBt ester of α-Fmoc-Boc-Lys prepared by pre-activation of a solution of α-Fmoc-Lys (159mg, 0.339 mmol), HOBt (46mg, 0.339), TBTU (108mg, 0.339 mmol) and Hunig's Base (60ml, 0.339 mmol) in DMF (10ml) for a period of 30 min. The resin cloth was reacted at room temperature overnight with this preformed reagent.

The two pieces of resin cloth were then washed with DMF (x 3) to remove reagents, dichloromethane (x 6), and dried under vacuum.

Residual amine groups were acetylated using a solution of acetic anhydride (4ml), collidine (6ml) and 4-dimethyl-aminopyridine (2g) in acetonitrile (20ml) for 1hr at room temperature. The resin cloth was then washed with acetonitrile (x 3), dichloromethane (x 6), and dried under vacuum.

Final deprotection of the Fmoc group was achieved using a standard method utilising a solution of piperidine (10ml) in DMF (10ml) for 30 min at room temperature. Washing of the cloth with DMF (x 4) and dichloromethane (x 6), followed by drying under vacuum, gave the material ready for the final cleavage.

Cleavage of the trimers from the resin cloth:

The sample of resin cloth bearing Thr-Tyr-Ser was separately treated with TFA/H_2O (95:5, 30ml) and stored at room temperature overnight. The acid solution was removed from the cloth by filtration, was removed by evaporation below 40°C, and the sample freed from acid by azeotropy from toluene/dichloromethane (3 x). The mass recovery was essentially quantitative and the presence of the desired product was confirmed by hplc and ms analysis.

The sample of resin cloth bearing Lys-Tyr-Lys was separately treated with TFA/H_2O (95:5, 30ml) and stored at room temperature overnight. The acid was removed by evaporation below 40°C, and the sample freed from acid by azeotropy from toluene/dichloromethane (3 x). Again, the mass recovery was essentially quantitative and formation of the desired product was confirmed by hplc and ms analysis.

Preparation of a 27 component tripeptide library on resin cloth for biological screening:

Derivatisation with the first monomer (using α-Fmoc-ω-Boc-Lys, Fmoc-Ser-O-*t*-butyl ether and Fmoc-Leu):

Three samples of the above polypropylene resin cloth (each 210 x 150 mm, or 68.7 mmol total amine content), each having reaction zones indelibly identified in a 3 x 3 grid pattern of indicia, were derivatised separately by reaction in DMF solution for a period of 17hr at room temperature with 2,4-dichlorophenyl-α-Fmoc-aminoacyl-4-oxymethylphenoxyacetate (0.34 mmol or 5 times excess) derivatives of the amino acid monomers listed above. The resin cloth was washed with DMF solution (x 3) to remove reagents, dichloromethane (x 5) and was then dried at room temperature under vacuum for 15 min.

Residual amine groups were acetylated on the whole set of sheets using a solution of acetic anhydride (6ml), collidine (9ml) and 4-dimethylaminopyridine (3g) in aceto-nitrile (30ml) for 1hr at room temperature. The resin cloth

was then washed with acetonitrile (x 4) and dichloromethane (x 6), and dried under vacuum for 1hr.

Deprotection of the Fmoc group was achieved using a standard method utilising a solution of piperidine (15ml) in DMF (15ml) for 30min at room temperature. Washing of the cloth with DMF (x 4) and dichloromethane (x 6), followed by drying of the cloth under vacuum, gave the material ready for the next coupling.

Derivatisation with the second monomers (using FmocTyr-O-*t*-butyl ether, FmocSer-O-*t*-butyl ether and Fmoc Phe):

The original sheets were divided into three columns, and each set of three columns of three reaction zones was reacted separately with the second monomer. Each of the nine monomerically linked pieces of resin cloth in three columns was combined in one vessel and treated with a five-fold excess of a solution of the HOBt ester of the above Fmoc-amino acid preformed from Fmoc-amino acid (0.343 mmol, 5 times excess), HOBt (46mg, 0.343mmol), TBTU (108mg, 0.343 mmol) and Hunig's Base (60ml, 0.343 mmol) by reaction in DMF (10ml) for a period of 30min. This was then reacted at room temperature overnight with the samples of resin cloth. The pieces of resin cloth were then washed to remove reagents using DMF (x 3), dichloromethane (x 5), and dried under vacuum at room temperature for 15 min.

Residual amine groups were acetylated using a solution of acetic anhydride (6ml), collidine (9ml) and 4-dimethyl-aminopyridine (3g) in acetonitrile (30ml) for 1 hr at room temperature. The resin cloth was then washed with acetonitrile (x 4), dichloromethane (x 6), and dried under vacuum for 1 hr.

Deprotection of the Fmoc group was achieved using a standard method utilising a solution of piperidine (15ml) in DMF (15ml) for 30 min at room temperature. Washing of the cloth with DMF (x 4) and dichloromethane (x 6) followed by

drying of the resin cloth under vacuum gave the material ready for the final coupling.

Coupling of the third monomers (using FmocThr-O-t-butyl ether, α-Fmoc-ω-Boc-Lys and Fmoc-Gly):

The samples of resin cloth were divided into their individual portions by cutting in a direction orthogonal to the first cut. Each set of samples of resin cloth bearing dimeric units was further reacted separately with a sample of the HOBt-ester of Fmoc-amino acids as listed above, prepared by pre-activation of a solution of Fmoc-amino acid (0.343 mmol), HOBt (46mg, 0.343 mmol), TBTU (108mg, 0.343 mmol) and Hunig's Base (60ml, 0.343 mmol) in DMF (10ml) for a period of 30 min. The resin cloth was then reacted at room temperature overnight with this pre-activated reagent. Each individual piece of resin cloth was then washed to remove reagents with DMF (x 3) and dichloromethane (x 6), and dried under vacuum.

Residual amine groups were acetylated using a solution of acetic anhydride (6ml), collidine (9ml) and 4-dimethyl-aminopyridine (3g) in acetonitrile (30ml) for 1hr at room temperature. The resin cloth was then washed with acetonitrile (x 4), dichloromethane (x 6), and dried under vacuum.

Final deprotection of the Fmoc group was achieved using a standard method utilising a solution of piperidine (15ml) in DMF (15ml) for 30 min at room temperature. Washing of the cloth with DMF (x 4) and dichloromethane (x 6), followed by drying under vacuum, gave the material ready for the final cleavage.

Cleavage of trimers from the resin cloth:

Each sample of resin cloth bearing an individual trimeric unit was separately treated with TFA/H_2O (95:5, 2ml) and stored at room temperature overnight. The cloth was separated by filtration, and washed with dichloromethane (2ml x 12), methanol (2ml x 4), and the washings combined. The acid was removed by evaporation below 40°C, and the sample freed from

acid by azeotropy from toluene/dichloromethane (3 x). The mass recovery was essentially quantitative and formation of the desired products was confirmed by hplc and ms analysis in comparison with an authentic sample. Results are given below:

Entry number	Structure	Expected m/e	Found m/e
1	ThrTyrLys	410.46	411.0
2	LysTyrLys	437.56	438.2
3	GlyTyrLys	366.46	367.3
4	ThrPheLys	394.46	395.6
5	LysPheLys	421.56	422.5
6	GlyPheLys	350.46	351.7
7	TheSerLys	334.46	335.0
8	LysSerLys	361.46	362.6
9	GlySerLys	290.36	291.5
10	ThrTyrSer	369.36	370.0
11	LysTyrSer	396.46	396.9
12	GlyTyrSer	325.36	326.6
13	ThrPheSer	353.36	354.1
14	LysPheSer	380.46	381.4
15	GlyPheSer	309.36	309.8
16	ThrSerSer	292.90	294.2
17	LysSerSer	320.36	321.3
18	GlySerSer	249.26	250.4
19	ThrTyrLeu	395.46	395.8
20	LysTyrLeu	422.56	423.3
21	GlyTyrLeu	351.46	352.1
22	ThrPheLeu	379.46	380.1
23	LysPheLeu	406.56	407.1
24	GlyPheLeu	335.46	336.6
25	ThrSerLeu	319.36	320.6
26	LysSerLeu	346.46	347.5
27	GlySerLeu	275.36	276.1

Preparation on resin cloth of a 1677 component library for biological screening:

Three sheets of the polypropylene resin cloth described above of dimensions 210 x 297mm were indelibly marked with a pattern of indicia (43 columns and 39 rows), ready for the coupling of the first monomers. Analysis revealed the presence of 2.18 nmol/mm^2 of amine groups.

Functionalisation of the sheet:

Each column of polypropylene resin cloth was divided from the original sheet, and separately functionalised by treatment with an individual Fmoc protected amino acid derivative pre-activated as its 2,4-dichlorophenyl 4-(oxymethyl)phenoxy acetate as described above in a solution of DMF (0.5ml) and pyridine (10ml) at room temperature overnight. The cloth strips were washed with DMF (x 4) and dichloromethane (x 5) and dried at 40°C for 30 min. Acetylation of residual amine functionality was carried out as described above.

Deprotection of the amine groups was also carried out as described above.

Coupling of the second monomer:

The complete set of columns of the original polypropylene resin sheets was assembled into one block and cut into individual pieces. Each set of pieces from individual rows was then coupled with a second Fmoc-protected monomeric unit, pre-activated as its 2,4-dichlorophenyl 4-(oxymethyl)phenoxy acetate ester as described above. Upon completion of reaction, these were washed, acetylated and, finally, the Fmoc protection group was removed as described above.

Coupling of the third monomer:

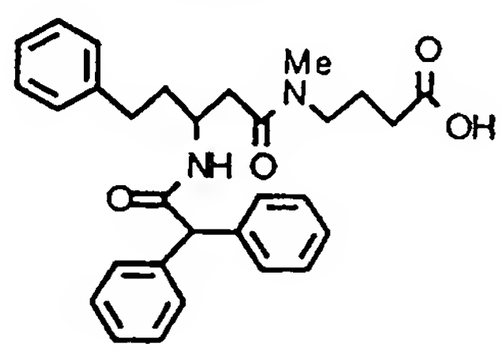
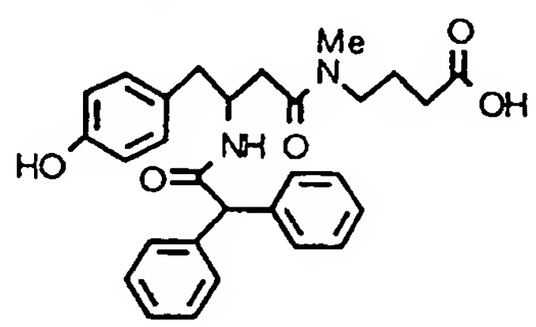
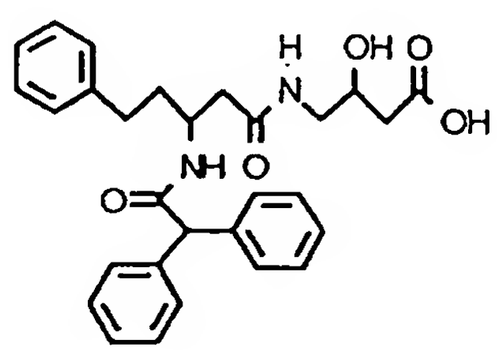
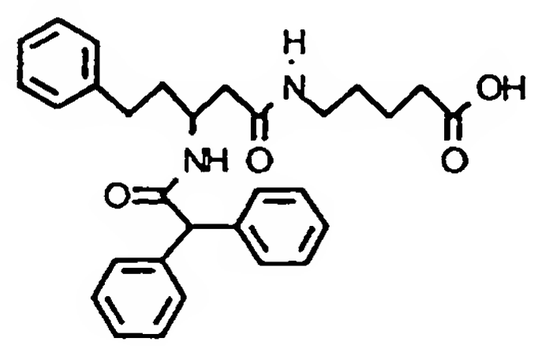
The complete set of 1677 individual dimeric amine derivatives was reacted with diphenylacetyl chloride (2.31g, 0.1 mol) and Hunig's Base (3.5ml, 0.1 mol) in DMF (96.5ml) at

room temperature overnight in smaller subsets. Each set of polypropylene resin cloth pieces was washed with DMF (x 3), dichloromethane (x 4) and dried at 40°C (30 min) under vacuum.

Cleavage of individual trimeric products from the polypropylene cloth:

Individual labelled resin cloth pieces were separated and treated with TFA/H_2O (95:5, 50ml) at room temperature overnight. Each was then washed with acetonitrile/water (1:1, 3 x 100ml), the washings combined and evaporated under vacuum centrifugation. After cleavage from the resin cloth support, individual trimeric products were identified by the indicia marked thereon. A subset of these was examined by both hplc and mass spectrometry and, in the cases examined, confirmed the formation of the desired compounds.

A subset of typical analytical data for compounds examined by mass spectrometry is given below:

Indicia	Structure	Expected m/e	Found m/e
B3412		487.59	487.00
B2412		458.54	475.0*
C3408		488.57	489.00
A3408		486.59	505.1*

* MNH^{4+}

CLAIMS

1. A method of making a library of compounds, which method comprises the following steps:

(a) individually identifying a plurality of discrete reaction zones defined on laminar solid support material;

(b) charging each of said reaction zones with a starting material;

(c) sub-dividing the reaction zones into at least two initial batches;

(d) applying at least two different reagents, one to each of the reaction zones in each initial batch, and recording the identity of those reaction zones to which each of said different reagents is applied;

(e) subjecting all reaction zones to reaction conditions which promote reaction to completion;

(f) further sub-dividing the reaction zones into at least two alternative batches;

(g) applying at least two different reagents, one to each of the reaction zones in each alternative batch, and recording the identity of those reaction zones to which each of said different reagents is applied;

(h) subjecting all reaction zones to reaction conditions which promote reaction to completion, and

(i) repeating steps (f) to (h) inclusive from zero to n times, as desired.

2. A method as claimed in claim 1 wherein the reaction zones are defined on a sheet or a plurality of sheets of material.

3. A method as claimed in claim 2 wherein each sheet is charged with a different starting material in step (b).

4. A method as claimed in any one of claims 1 to 3 wherein the compounds obtained at the end of step (i) are linked to said laminar solid support material by chemical linker groups which are cleavable by acidic, basic, hydrogenolytic or other chemical reagents, by light induced cleavage, or by combinations of these.

5. A method as claimed in any preceding claim wherein the applied reagents are selected from the group comprising amino acids, nucleotides, sugars, naturally-occurring and synthetic heterocycles, lipids, and combinations thereof.

6. A method as claimed in any preceding claim wherein the reaction zones are identified by application of indicia selected from the group consisting of numbers, letters, symbols or colours in a coded combination, Smiles strings, bar-codes, chemical structures, marked or printed punched cards; ultraviolet-readable systems and electro-magnetically readable devices.

7. A method as claimed in claim 1 comprising:

providing a plurality of sheets of material to which the compounds may be releasably bound, each sheet bearing indicia identifying individual reaction zones defined on said sheet arranged in rows and columns in a two-dimensional array;

treating each sheet with respective first reagents to bind respective first constituents to each of the individual reaction zones on the sheets;

superposing the sheets to form a block and repeatedly subdividing the block parallel to one of its faces to form a plurality of stacked strips derived from respective divided sheets, each strip bearing one of said rows of reaction zones;

treating each of the stacks of strips so formed with respective second reagents to bind respective second constituents to the first constituents on the strips;

reassembling the treated stacks of strips to reform the block and dividing the block parallel to second face thereof disposed at right angles to said first face so that each strip is divided into stacks of individual reaction zones, and

treating each of the stacks of reaction zones so formed with respective third reagents to bind respective third constituents to the growing substrate already bound on the reaction zones.

8. A method as claimed in claim 7 wherein the individual reaction zones are reassembled and further sub-divided prior to treatment with respective fourth reagents to bind respective fourth constituents to the growing substrate already bound on the reaction zones, and optionally repeating the re-combination, sub-division and treatment steps as many times as desired.

9. A method of making a library of compounds substantially as described herein with reference to the examples.

10. A method of preparing a paper support material for use in the synthesis of chemical compound libraries, which method comprises:

(a) linking cellulose with a compound which is selected from the set consisting of an amine precursor or a compound having a protected amine group;

(b) in the case of an amine precursor, generating the free amine and then protecting it with a conventional amino protecting group;

(c) incorporating the amine-functionalised cellulose into a paper sheet by mixing with paper fibre and forming into sheets, and

(d) reacting the paper sheets obtained from step (c) above with an amino deprotecting reagent to provide free amine groups on the paper sheets.

11. A method of preparing a laminar resin support material for use in the synthesis of chemical compound libraries, which method comprises affixing a layer of particulate functionalised solid support resin material to a porous inert laminar material.

12. A method of preparing a laminar resin support material as claimed in claim 11 wherein the layer of particulate functionalised solid support resin material is sandwiched between two layers of porous inert laminar material.

13. A laminar resin support material when prepared by the method of claim 11 or claim 12, in the form of a cloth.

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Patents Act 1977 CORRECTED **Examiner's report to the Comptroller under Section 17** **(The Search report)**	Application number GB 9423332.7
Relevant Technical Fields (i) UK Cl (Ed.N) C3H (HA3, HB4A, HB4B) (ii) Int Cl (Ed.6) C07K 1/04; C07H 21/00	Search Examiner MR C SHERRINGTON Date of completion of Search 20 FEBRUARY 1995
Databases (see below) (i) UK Patent Office collections of GB, EP, WO and US patent specifications. (ii) ONLINE DATABASES: WPI, CLAIMS, DIALOG/BIOTECH	Documents considered relevant following a search in respect of Claims :- 1 TO 9

Categories of documents

X: Document indicating lack of novelty or of inventive step.

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&: Member of the same patent family; corresponding document.

Category	Identity of document and relevant passages	Relevant to claim(s)
A	WO 92/10092 A1 (AFFYMAX TECNOLOGIES N.V) whole document, especially Figures 1, 2	1
A	WO 93/06121 A1 (AFFYMAX TECNOLOGIES N.V) whole document, especially Figures 1, 2	1
A	Bioorg. Med. Chem. Lett. 1993, 3(3), 425-430 Strategies and Techniques in Simultaneous Solid Phase Synthesis based on the ...	1
A	Drug Dev. Res. 1994, 33(2), 174-188 Recent Advances in the Generation of Chemical Diversity Libraries	1

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